Module designation	*: **Microbiology***
Semester(s) in which the module is taught	*: odd/II*
Person responsible for the module	1. *Dr. Esti Utarti, S.P., M.Si.,* 2. *Dr. Drs. Sutoyo, MSi.,* 3. *Drs. Rudju Winarsa, M.Kes.* 4. *Drs. Siswanto.M.Si* 5. *Kahar Muzakhar,Ph.D*
Language	: *Bilingual*
Relation to curriculum	: *Compulsory/~~elective~~/~~specialization~~* Chemical *UNEJ*
Teaching methods	*: Lecture- Discussion, lab works, case method, Presentation*
Workload (incl. contact hours, self-study hours)	*(Estimated) Total workload: 136 hr* a. *Lecture-Discussion*: *68 hr* b. *Laboratory work : 34 hr* c. *Case method: 11.33 hr* d. *Topic Presentation : 22.67 hr*
Credit points	*: 3 credits or 4.53 ECTS*
Required and recommended prerequisites for joining the module	*: Fundamental Biology*

Module objectives/intended learning outcomes	*Knowledge: able to analyze the principles of molecular biology, cells, and organisms (LO 3)* ● *describing the principles of microorganism structure and diversity of microorganisms (LO 3.a)* *Skills: able to implement biological concepts in laboratory work and/or field studies independently and/or in group (LO 6)* ● *practicing laboratory work in groups on the technique of observing the morphology of various microorganisms (LO 6.a)* ● *using software and/or basic instruments to create microbia cluster (LO 6.b)* *Competencies: able to internalize norms and ethics based on Pancasila in working independently or in groups (LO 1)* ● *working in team works and individually on microbiology concepts discussion (LO 1.b)* *Key question: what learning outcomes should students attain in the module?* *E.g. in terms of* - *Knowledge: familiarity with information, theory and/or subject knowledge* *Skills: cognitive and practical abilities for which knowledge is used*

	- *Competences: integration of knowledge, skills and social and methodological capacities in working or learning situations*[1] *E.g.: "Students know that/know how to/are able to…"*
Content	*This course describes the principles of microorganism structure and diversity of microorganisms that cover describe the principles of microorganism structure and diversity of microorganisms that covers scope and development of microbiology, basic concepts of microbiological analysis, the cell structure of microorganisms in prokaryotes and eukaryotes, non-cellular microorganisms of viruses, prokaryotic microbial diversity of proteobacteria, prokaryotic microbial diversity of non-proteobacteria, prokaryotic microbial diversity of Gram-positive bacteria, prokaryotic microbial diversity of archaebacteria, eukaryotic microbial diversity of yeast, eukaryotic microbial diversity of fungi, eukaryotic microbial diversity of algae, and eukaryotic microbial diversity of protozoa.* *There is also implementation of biological concepts in laboratory work and/or field studies independently and/or in groups through* ***Case Method****: microbial diversity based on specimen morphological identification of origin from ecosystems in the tropics through practical work. It is assigned in* ***individual or teamwork*** *covering 1) Preparing sterile medium, 2) Sampling of isolate source material and measurement of environmental data,*

[1] Cf. European Commission: Proposal for a Recommendation of the European Parliament and the European Council on the establishment of the European Qualifications Framework for lifelong learning, COM(2006) 479 final, 2006/0163 (COD), Brussels 05/09(2006.

	3) Microbial isolation technique from isolate source, 4) Observing colony morphology of bacteria, 5) Microscopic observation of bacteria, 6) Observing colony morphology of actinomycetes, 7) Observing microscopic morphology of actinomycetes in the culture slide, 8) Observing microscopic morphology of molds and yeast in the culture plate, 9) Observing microscopic morphology of molds and yeast in the culture slide, 10) Observing morphology of algae and protozoa ***Data analysis using Microsoft Excel and Statistic Software*** *for microbial identification base on clustering morphology characteristic of microbial isolates, 8) Results and Discussion of Project, and 9) writing project report as* ***a scientific article draft*** *and* ***presentation*** *in class* *The description of the contents should clearly indicate the weighting of the content and the level.*
Examination forms	1. *Multiple choice (5%)* 2. *Essay test (20%)* 3. *Paper review (%)* 4. *Topic presentation (15%)* 5. *post test (5%)* 6. *Response test (10%)* 7. *Equipment software observation(5%)* 8. *Activity observation (10%)* 9. *Final Report (10%)* 10. ~~*Report presentation (10%)*~~

	e.g. oral presentation, essay, etc.
Study and examination requirements	*: passing grade 70%* *Requirements for successfully passing the module*
Reading list	1. *Cappuccino, J.G. and Welsh, C. 2020. Microbiology: A Laboratory Manual. Pearson.* 2. *Kim, B.H and G.M. Gadd. 2008. Bacterial Physiology and Metabolism. Cambridge University Press. Cambridge* 3. *Madigan, M.T, J.M Martinko and J. Parker. 2019. Biology of Microorganisms. Prentice-Hall.* 4. *Brenner, D.J., N. R. Krieg and J.T. Staley. Bergey's Manual of Systematic Bacteriology 2nd edition part A. Springer.* 5. *Brenner, D.J., N. R. Krieg and J.T. Staley. Bergey's Manual of Systematic Bacteriology 2nd edition part B. Springer.*

Credits to ECTS conversion formula 1 SKS TM = 50min T+60min TS+60min M (170 minutes) x 16 weeks = 45.33 Hours 1 SKS Practice = 170 min. 1 ECTS = 29.99 hours 1 Credit = 1.51 ECTS

PRACTICAL MODULE

COURSE NAME : MICROBIOLOGY
COURSE CODE : MAB 1201
ACADEMIC YEAR : 2021/2022

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DEPARTMENT OF BIOLOGY
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THE UNIVERSITY OF JEMBER
2022

PREFACE

This Microbiology Practical Modul is a guidebook for working in the Laboratory for students who take the General Microbiology practical. The main objective is to introduce the basic concepts of microbiology along with basic laboratory techniques and procedures.

Therefore, mastering these things well will greatly help students in studying microbiology, further will be able to explore various fields of applied microbiology. The availability of books that are thought of and the readiness of the assistants / lecturers is very supportive to achieve the intended goal. Likewise, laboratory advice and infrastructure must also be ready to help achieve goals.

On this occasion we would like to thank all parties who have helped in the content of the material and who have helped in the technical implementation until the realization of this book. Suggestions and criticisms from users of this book are highly expected so that subsequent publications can be added/improved.

Jember, February 25 2021

The Nurturing Team of Course

TABLE OF CONTENTS

PRACTICE RULES

Every microbiology practicum, practice will always be related to microbes. To prevent unwanted things, the practitioners must obey the predetermined rules and carry out the instructions given by the assistant. Work in the Hygiene Microbiology Laboratory is a major factor both for clothing, equipment and so on.

Work in the Hygiene Microbiology Laboratory is a major factor both for clothing, equipment and so on. The rules of conduct that must be observed by practice are as follows:

1. Students must be present on time.
2. Students must have learned the theory of practicum which will be carried out before the practicum takes place, because before the practicum, a test will be held first.
3. The tool to be used must be clean and intact. If there is a malfunction of the tool, you should immediately report it to the assistant, and in practice it is not allowed to repair the damaged tool yourself.
4. Practices should be held accountable for tools that are tampered with, solved, or lost.
5. Reduce conversing during the practicum, do not allow eating, drinking, smoking in the practicum room unless it has something to do with the practicum event.
6. For those who are unable to attend / unable to participate in the practicum, there must be a written notice with realistic reasons and must do an practical remedial on the day specified by the assistant by paying administrative fees in accordance with applicable rules. If three consecutive times the students does not participate in the practicum without notice, then the students cannot participate in the next practicum/cancel.
7. Any results of the observation of practicum results must be carefully recorded.
8. Any practice is not allowed to conduct experiments outside the practicum event.

MEETING 1. ASEPTIC AND STERILIZATION TECHNIQUES

PURPOSE

Student understand how aseptic techniques and how to sterilize.

PRINCIPLE

This aseptic action aims to reduce or eliminate microorganisms found on the surface of living objects or inanimate objects. This action includes antiseptics and sterilization. Antiseptics are materials that can kill or inhibit the growth of microbes. Before breeding microbes the first thing to do is to carry out sterilization of the substrate and tools. This is done to remove microbes from contaminants, so that the material and all laboratory equipment must be sterile. To sterilize tools and materials using sterilization techniques.

Sterilization is an attempt to free a tool or material from all sorts of life forms, especially microbes. In the laboratory, tools and media are always used in a sterile state. This is so that other unwanted microbes do not grow.

The speed of microbial death during sterilization depends on the type of microbe and its environmental factors. At the time of sterilization it is necessary to pay attention to the type of material and the nature of the material and sterilized tools. In addition, the hydration factor of sterilization is also considered.

1. Dry sterilization

1.1. Sterilization by fingering (fire)

This method is mainly used for sterilization of inoculation needle equipment, object glasses, tweezers, spatels, mouths of culture tubes and so on. The trick, by burning the tool on the fire of the lamp ethyl alcohol or gas until incandescent, to cool the tool is put in 70% alcohol then burned again to remove the remaining alcohol.

1.2. Sterilization with hot air (oven = hot air sterilization)

This tool is used to sterilize glass tools, such as Erlenmeyer flask, Petri dish, test tube, pipette, and others. Sterilization is carried out by heating at a temperature of 170-180oC for 2 hours. before sterilization glassware is put in a container made of metal/iron or can also be wrapped in doorslag paper. It is necessary to pay attention to the duration of sterilization depending on the number of tools and their resistance to heat. After completion of sterilization the electricity flow is cut off and leave the appliance to cool. Then the sterilized tools just now were taken away.

2. Wet sterilization

2.1. Sterilization with hot water vapor (Tyndallization= Arnold Steam Sterilizer = multistage sterilization)

This sterilizer is used for materials/media that are resistant to high heat, for example: milk, sugar, and so on. In common cells In general, microbial vegetative cells die at a temperature of 100oC in a humid state. Sterilization is carried out as follows: at first the place where the water is filled and heated to boiling. Then the material to be sterilized is laid out on the goose contained on the water. The place where the ingredients will be sterilized beforehand is tightly closed. The first time the material is sterilized at 100^{o}C for 30 minutes to kill microbial vegetative cells, then the material/medium that has been sterilized first degree, incubated at room temperature for 24 hours, to provide an opportunity for spore growth. Next, the second sterilization is carried out at a temperature of 100^{o}C at 30 minutes, to ensure the sterility of the material, sterilized again at the same temperature and time.

The use of sterilization by this method is likely to occur failures caused due to:

a. Sterilized substrate is not suitable for spore germination

b. Spores of anaerobic bacteria that may be present, do not grow in a medium that is directly related to hot air.

2.2. Sterilization with pressurized hot water vapor (Autoclave)

The tool is used for sterilization of the substrate. This method is the best sterilization method compared to other methods. Sterilized material is an undamaged material or tool with heating and high pressure. The sterilization process is carried out at high pressure. The sterilization process is carried out at a pressure of 15 lbs at a temperature of 121ºC for 15-20 minutes.

There are two types of Autoclaves:

a. Autoclave with heating devices using electricity

b. Autoclave with heating device using a regular stove

In principle the way it works is the same. The tool or material to be sterilized is prepared in advance and tightly closed. For pipettes as good as tightly wrapped. At first the autoclave is filled with water and then a sash is installed. The tool or material is placed on the gooseng. The tap for moisture removal is opened. After the water boils, the tap is closed. The temperature will rise to 121ºC and the pressure rises to 2 atmospheres.

The temperature and pressure are maintained as above for 15-30 minutes. When the sterilization has been completed, the electricity is cut off or the stove is turned off, wait until the autoclave cools down. The tap is opened after the autoclave has cooled down, then the lid of the autoclave is opened. Materials and tools that have been sterile are taken. For a substrate that is still hot, it is cooled at room temperature. For this tool must be dried in the oven (sterilization of hot water).

3. Sterileization with filtering

This method is used for thermolybilizing materials, for example, blood serum, toxins, vitamins, amino acids, sugars, Na_2CO_3, etc. For sterilization of the solution is carried out filtering using a bacterial filter, a vacuum pump is used for suction, so the solution will pass through the filter smoothly.

3.1. Berkefeld filter = diatomaeus earth/infusorial earth arth

Filter with elem filter scattered from the ground diatoma with porosity V= veil = coarse; N= normal, and W= wenig = smooth.

3.2. Chamberland Filter = porcelain

This filter tool is made of fine porcelain, this filter has some porosity L1-L13.

3.3. Sietz Filter

This is a non-rusty metal filter device, equipped with interchangeable asbestos-cellulose filters and suction flasks which are connected by a vacuum pipe. The pressure of the vacuum pump used is 20-30 mmHg which is enough to accelerate filtration without causing foam to form on the filtered medium. All filters, namely anti-rust vessels and suction flasks, must first be sterilized before use.

3.4. Air filter

To obtain dust-free air and microbes, HEPA (high-efficiency portiquate water) is often used which is combined with laminar airflow.

4. Sterilization with Disinfectants

Disinfectants are chemicals, usually in the form of solutions that have the property of being able to kill microbial vegetative cells, but not kill endospores. Examples of disinfectants include H_2O_2, O_3, $HgCl_2$ 1%, $CaCl_2$, formalin 4% and Methanol 50% commonly used to sterilize grains. After sterilization with such a solution, the seeds must be rinsed with sterile aqueducts.

5. Pasteurization

This method is used for solutions that are easily damaged when exposed to too high heat, for example: milk. Pasteurization is carried out by heating the material at a temperature of 63ºC for 30 minutes and then cooling down quickly. Or heated at a temperature of 71.6 – 80oC for 15-30 seconds

then cooled.

Preparation of tools for sterilization

MATERIALS

Equipments

Petri dishes, tweezers, brown paper, test tube, cotton

PROCEDURE

1. Wrapping tools (Petri dishes, tweezers) with brown paper
2. Covering the test tube with a cotton

Sterilization with Autoclave

MATERIALS

Media

Media in the flask and test tube

Equipments

Autoclave, Petri dishes, tweezers

PROCEDURE

1. Fill the autoclave with drains until the heater is all submerged.
2. Inserting tools or media
3. Close the autoclave door and tighten the bolts.
4. The air pressure regulator is closed.
5. After the water boils, the air pressure regulator is opened.
6. When the smoke runs out, cover the air pressure and leave until the needle shows a figure of 1.5 kg f/cm2 and is constant
7. Constant figure 1.5 kg f/cm2 for 15 minutes.
8. Open the air regulator for the needle to go down.
9. When the number 0 opens the lid of the autoclave.

Sterilization with oven

MATERIALS

Equipments

Oven, wrapped glass ware

PROCEDURE

1. Turn on the oven/incubator.
2. Set the temperature to a temperature of 105 ^{o}C.
3. Wrap the tool to be sterilized with paper.
4. When the oven temperature is 105 ^{o}C insert the appliance.
5. Ventilate for 60 minutes.

Sterilization directly with fire

MATERIALS

Equipments

Ose needle, Bunsen lamp

PROCEDURE

1. Preparing the ose needle
2. Incandescent is incandescent to smoldering red. So that the heat from the metal does not kill the tested microbes, it can be aerated near the fire or attached to the edges of the sterile medium

Engineering in The Working Area

MATERIALS

Reagents

70% alcohol solution

Equipments

Bunsen lamp, test tube, Petri dish, ose needle

PROCEDURE

1. Cleaning the work area (table) with 70% alcohol.
2. Hand sanitizer (all jewelry and watches are removed) with 70% alcohol
3. Turning on the Bunsen lamp in the work area for 15 minutes of air effort around the sterile Bunsen
4. After all is sterile, then microbiological work (isolating microbes) is carried out aseptically. Opening and closing the test tube/Petri dish is done near the fire.
5. Sterilization of the ose needle is used by burning the Bunsen lamp until it is red, then cooled first before hitting the isolate by scraping it near the fire.
6. The process of taking isolates in test tubes aseptically is presented in Figure 1.
7. Aseptic techniques number 1 and 2 are carried out before and after work.

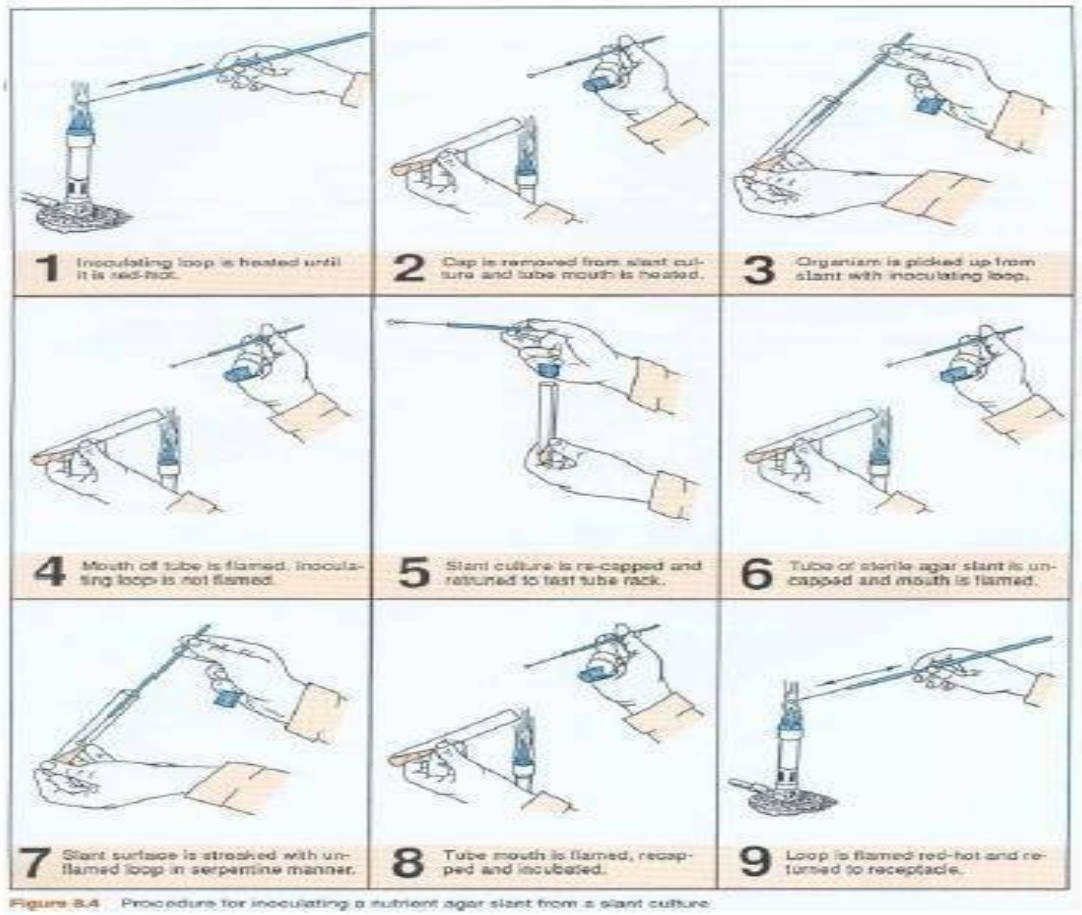


Figure 8.4 Procedure for inoculating a nutrient agar slant from a slant culture

Figure 1. Aseptic techniques

MEETING 2. MEDIUM AND HOW TO PREPARE MEDIUM

PURPOSE

Student understand the types of media and how to make media

PRINCIPLE

Medium is an ingredient consisting of a mixture of nutrients used to grow microbes. In addition, this medium is also used for isolation, propagation, testing physiological properties, counting the number of microbes, etc. A good medium should contain all the substances needed for the life of microbes, including organic compounds (proteins, carbohydrates, fats), minerals and vitamins.

A. Media terms

In order for microbes to grow well, the substrate must meet the following conditions:

- Must contain nutrients that are easy to use microbes
- Must have osmosal pressure, crushing voltage and corresponding pH
- Must not contain toxins
- Must be sterile

B. Classification of mediums

1. Based on the materials used:

➢ Natural medium or substrate

This medium consists of natural ingredients such as:

Cider, carrots, rice, corn, blood, milk, meat and other natural ingredients.

➢ Semi-natural medium

This medium comes from natural ingredients coupled with chemical compounds such as potato dextrose agar (PDA), bean sprouts extract agar (TEA), malt extract agar (MEA), etc.

➢ Artificial media or synthetic media

This medium consists of chemical compounds whose composition and quantity have been determined e.g. czapeks dox agar (CDA), sabouraud dextrose agar (SDA), etc.

2. Based on its usefulness

➢ General media

This media is overgrown by microbes in general, namely many types of microbes that grow on this medium, for example agar-agar (NA), potato dextrose agar (PDA), agar bean sprouts extract (TEA), etc.

➢ Selective media

This medium is structured in such a way, that only certain types of microbs can live, for example *Salmonella Shigella* Agar (SSA), brillian green lactose broth (BGLB).

➢ Differential media

This medium is used to distinguish the types of microbes from each other, due to the presence of a formed reaction or characteristic. This reaction occurs because microbes are able to decompose any of the ingredients in the medium, e.g. eosin methylen blue agar (EMBA), blood agar (BA), etc.

➢ Enrichment media

This medium is used to grow certain organisms, before use in fermentation processes. The goal is to activate microbes, e.g. AEC for yeast.

➢ Medium tester (Testing medium)

Media that have a certain structure, are used to test for vitamins, amino acids, antibiotics, e.g. PPA (Phenyl Alanine Agar) etc.

➢ Special media

A medium for determining the type of growth of microbes and their ability to make certain chemical changes.

3. Based on the physical

➢ Solid media (agar)

This medium is given bacto agar, so that at room temperature the medium hardens. The function of bacto agar is to compact the medium and not as a source of nutrients. Example: nutrient agar

➢ Liquid medium (broth)

This medium is not given agar bacto agar so that it is liquid. Example: nutrient broth.

C. How to Create the Medium

The creators of a medium consisting of several materials are as follows:

1. Mix the ingredients: the salt is dissolved in aquadest, then heated in a water bath so that the solution is homogeneous.
2. Filtering media: some media sometimes need to be filtered; as a filter can be used filter paper, cotton wool, or casa cloth. For agar/gelatine media, filtering is carried out as long as the media is still hot.
3. Determining and regulating pH: the pH determination of liquid media can be used with universal indicator paper, block comparator, or pH meter.
4. Inserting the substrate in certain places: before sterilization, the substrate is inserted into a tube of 5 mL (for inclined media) or 10 mL (for upright media). Or a bottle of Erlenmeyer flask (its volume should not exceed 2/3 of the volume of its container). The tube/flask/bottle is further corked with a cotton swab. This plug must be tight and strong, the cotton part is wrapped in covering paper (parchment paper) so that it does not get wet when sterilized.
5. Sterilization of media: how to sterilize depending on the type of substrate, in general the substrate is sterilized with an autoclave at a temperature of 121^{o}C for 15 minutes; sugar-containing medium, milk, can be sterilized by filtering. If the substrate is in a sealed bottle, it should be tried that the lid of the bottle is not tightly closed during sterilization; only after exiting the autoclave, the bottle cap is tightened.

PREPARATION OF A NUTRIENT AGAR MEDIUM (NA)

MATERIALS

Media

Nutritional Composition of Agar-agar Nutrient Broth 23 g/L, Bacto Agar 15 g/L

Equipments

Analytical scales, spatulas, aluminium foil, markers, Beaker glass, test tubes, measuring cup, Erlenmeyer flask, Petri disk, heater and stirrer, autoclave

PROCEDURE

1. Weighing the ingredients of Nutrient Broth and Bacto Agar for a 50 mL medium
2. Put the ingredients gradually in it A 100 mL glass cup containing 50 mL of aquadest solution while stirring until homogeneous.
3. Insert 3 test tubes of 10 mL each and 4 test tubes of 5 mL each then plug them in when sterile
4. Sterilize with an autoclave (121^{o}C 15 lbs) for 15 minutes.
5. A total of 3 test tubes containing 10 mL of NA after being removed from the autoclave and a temperature of about 50^{o}C were poured aseptically on the Petri sterile plate for the preparation of the insulating medium.

6. A total of 4 test tubes containing 5 mL of NA after being removed from the autoclave are placed on an inclined board so that it can be tilted.

PREPARATION OF A NUTRIENT BROTH MEDIUM (NB)

MATERIALS
Media
Composition of Nutrient Broths: Nutrient Broth 23 g / L
Equipments
Analytical scales, spatulas, aluminum foil, markers, Beaker glass, test tubes, measuring cup, Erlenmeyer flask, Petri disk, heater and stirrer, autoclave

PROCEDURE
1. Weighing the ingredients of the Nutrient Broth for a 10 mL medium
2. Put the ingredients gradually into it a 50 mL glass cup containing 10 mL of aquadest gradually while stirring until homogeneous.
3. Insert two 5 mL test tubes and then plug them in when sterile
4. Sterilize with an autoclave (121^{o}C 15 lbs) for 15 minutes.

PREPARATION OF A POTATO DEXTROSA AGAR MEDIUM (PDA)

MATERIALS
Media
PDA composition: potato extract 200 g/L, dextrose 10 g/L, bacto Agar 15 g/L
Equipments
Analytical scales, spatulas, aluminum foil, markers, Beaker glass, test tubes, measuring cup, Erlenmeyer flask, Petri disk, heater and stirrer, autoclave

PROCEDURE
1. Weighing Potatoes for 50 ml of substrate, peeled and sliced into small pieces, then boil with 1 liter of water for 1 hour.
2. Strain and separate the pulp and filtrate
3. Put dextrose and bacto agar to fit the composition into the filtrate and stir over the water bath until it dissolves completely.
4. Insert 3 test tubes of 10 mL each and 4 test tubes of 5 mL each then plug them in when sterile
5. Sterilize with autoclave (121^{o}C 15 lbs) for 15 minutes.
6. A total of 3 test tubes containing 10 mL of PDA after being removed from the autoclave and a temperature of about 50^{o}C were poured aseptically on the petri sterile plate for the preparation of the insulating medium.
7. A total of 4 test tubes containing 5 mL of PDA after removal from the autoclave are placed on an inclined board so that the soil layer can be tilted.

PREPARATION OF A POTATO DEXTROSA BROTH MEDIUM (PDB)

MATERIALS
Media
PDA composition: potato extract 200 g/L, dextrose 10 g/L
Equipments

Analytical scales, spatulas, aluminum foil, markers, Beaker glass, test tubes, measuring cup, Erlenmeyer flask, Petri disk, heater and stirrer, autoclave

PROCEDURE

1. Make the media like point 3 without using Bacto agar 20 mL on a 100 mL Beaker glass
2. Insert 2 test tubes of 5 mL and 1 Erlenmeyer flask respectively 50 mL of 10 mL is then plugged in when sterile
3. Sterilize with an autoclave (121^{o}C 15 lbs) for 15 minutes.

PREPARATION OF A YEAST MALT EXTRACT AGAR MEDIUM (YMA)

MATERIALS

Media

Composition of Medium: yeast extract 4 g/L, glucose 4 g/L, maltose 10 g/L, bacto agar 15 g/L

Equipments

Analytical scales, spatulas, aluminum foil, markers, Beaker glass, test tubes, measuring cup, Erlenmeyer flask, Petri disk, heater and stirrer, autoclave

PROCEDURE

1. Weighing material for 50 mL media
2. Put the ingredients gradually in it A 100 mL glass cup containing 50 mL of aquadest gradually while stirring until homogeneous.
3. Put it in five 10 mL test tubes and then plug it in when sterile
4. Sterilize with an autoclave (121^{o}C 15 lbs) for 15 minutes.

PREPARATION OF A CARROTS MEDIUM

MATERIALS

Media

Carrots

Equipments

Analytical scales, markers, Beaker glass, test tubes with cotton plug, autoclave

PROCEDURE

1. Cut carrots with a triangular shape with a size of approximately 10 x 2 cm2.
2. The cut is placed inside the test tube and then the plug with when sterile
3. Sterilize with an autoclave (121^{o}C 15 lbs) for 15 minutes.

PREPARATION OF A PHYSIOLOGICAL SALT SOLUTIONS (0.85% w/v NaCl)

MATERIALS

Reagent

NaCl 8.5 g/ L

Equipments

Analytical scales, spatulas, aluminum foil, markers, Beaker glass, test tubes, measuring cup, heater and stirrer, autoclave

PROCEDURE

1. Weighing material for 50 mL of media
2. Put the ingredients gradually in it a 100 mL Beaker glass containing 50 mL of aquadest

gradually while stirring until homogeneous.
3. Insert five 9 mL test tubes and then plug them in when sterile
4. Sterilize with an autoclave (121ºC 15 lbs) for 15 minutes.

MEETING 3. CULTURING AND ISOLATION OF MICROBES

PURPOSE

Students can culture and isolate microbes from nature

PRINCIPLE

Microbes in nature consist of mold, yeast, bacteria as well as actinomycetes. Microbe can be beneficial or pathogenic in human life. To make it easier to study the types and properties of microbes, they must be isolated from their environment and planted in a medium suitable for their growth.

Isolation is a way to separate a certain microbe from its environment so that one type of microbes is obtained that is not mixed with other types of microbes. Microbe, which is no longer mixed with other types, is called a pure isolate.

The success of microbial isolation from a material is influenced by several factors , namely

1. Isolation techniques.
   The method of isolating, which is carried out aseptically, will ensure that the microbes obtained come from the samples used.
2. Types of media
   The type of media is very important to determine the microbes to be isolated. The nutritional content contained in the media varies according to the purpose of microbial isolation. Common media such as Nutrient Agar or Luria Agar allow various types of microbes to grow on these media. Specific media such as Medium Salmonella-Shigella Agar (SSA) and Humic acid-Vitamin (HV) are successively specific media for growing Salmonella bacteria and actinomycetes. The addition of antibiotics can accelerate the achievement of the goal of obtaining certain isolates The addition of antibiotics oxytetetracyline, penicillin, steptomycin or rose-bengal is used to isolate mold and yeast because it can inhibit the growth of bacteria. The antibiotics cycloheeximide, paramycin, and emdomycin inhibit the growth of mold and yeast so that they can be added to bacterial isolates.
3. Incubation conditions
   Incubation conditions such as temperature, aeration and pH are important factors for obtaining the desired microbial isolate. If the isolation is intended to obtain thermophilic microbes (live well at high temperatures) then incubation can be done at a temperature of at least 45 ºC. If you want to isolate anaerobic microbes, it can be done by storing petri dishes in an anaerobic jar. The addition of acidic or alkaline compounds can also be done to obtain a medium pH atmosphere suitable for microbe to be isolated.

The isolation technique largely determines the success of obtaining pure microbial isolates from nature. There are two ways of isolation to obtain pure culture, namely:

1. Streak *plate method*
   This method in principle is to scratch the suspension of the material containing microbes on the surface of the medium. After incubation, scratch marks will grow separate colonies
2. *Pour plate method*
   This method is basically inoculating the melting agar medium (temperature 40ºC) with a suspension of ingredients containing bacteria and pouring into a sterile petri dish. After incubation, it will be seen that the colonies are scattered on the surface so that the possible and expected come from one bacterial cell, so that they can be isolated.

MICROBIAL CULTURING AND ISOLATION

A. MICROBIAL ISOLATION FROM THE SOIL

MATERIALS

Media

Luria Agar Medium (LA)

Reagents

Physiological saline solution (0.85% b/v NaCl),

Equipments

Balance, Erlenmeyer flask, spatula, aluminium foil, measuring cup, incubator shaker, micro pipet, tips, test tube,

PROCEDURE

1. A soil sample of 10 grams was put into a 250 mL Erlenmeyer flask containing 90 ml of physiological saline solution (0.85% b/v NaCl) aseptically, then cornered to a homogeneous manner using an incubator shaker.
2. A dilution series is carried out by taking 1 ml of such suspense and introducing into 9 ml of physiological salts so that a dilution of 10-2 is obtained. This step is repeated until a dilution of 10-5 is obtained.
3. Microbial culturing is carried out by sprinkling. A total of 100 µl of dilution of 10-5 was poured evenly in a petri dish containing Luria Agar (LA) media and incubated at an incubator temperature of 30^{o}C for 1 – 3 days. Every day, observations are carried out for 4 days.
4. Observed and calculated the number of microbes colony that grow and have different characteristics between bacteria and mold.

B. MICROBIAL ISOLATION FROM THE AIR

MATERIALS

Media

Luria Agar (LA) media,

Equipments

Petri dish, incubator, colony counter, loop

PROCEDURE

1. Microbial isolation from the air is carried out by placing sterile Luria Agar (LA) medium on a Petri dish in an open condition for 10 minutes in a room under study.
2. After the cultures closed, it are incubated at an incubator temperature of 30^{o}C for 1 – 3 days.
3. Every day observations are carried out for 4 days.
4. Observed and calculated the number of microbial colonies that grow and have different characteristics between bacteria and mold.

C. ISOLATION OF MICROBES FROM FRUIT

MATERIALS

Sample

Grape, apple

Media
Luria Agar (LA) media
Reagents
Physiological saline solution (0.85% b/v NaCl)
Equipments
Incubator shaker, sterile test tube, micro pipet, tip, Erlenmeyer flask, petri dish

PROCEDURE
1. Microbial isolation from the fruit is carried out against microbes present on the surface of the fruit and endophytic microbes in the fruit.
2. Microbial isolation from the surface of the fruit is carried out using Grapes.
3. A sample of 10 grams of fresh Grapes was put into a 250 mL Erlenmeyer containing 90 ml of physiological saline solution (0.85% b/v NaCl) aseptically, then cornered using an incubator shaker for 10 minutes.
4. A dilution series is carried out so that a dilution of 10^{-5} is obtained.
5. Isolation of fruit endophytic microbes is carried out using apples.
6. Samples of apples as much as 10 grams were crushed using 50 ml physiological salt solution and put into Erlenmeyer flask 250 mL and added with 40 ml of physiological salt aseptically, then cornered using incubator shakers for 10 minutes.
7. A dilution series is carried out so that a dilution of 10-5 is obtained.
8. Microbial culturing is carried out by sprinkling.
9. A total of 100 µL suspension with dilution of 10^{-5} was poured evenly in a Petri dish containing Luria Agar (LA) media and incubated at an incubator temperature of 30ºC for 1 – 3 days.
10. Every day, observations are carried out for 4 days.
11. Observed and calculated the number of colony microbes that grow and have different characteristics between bacteria and mold.

PURIFICATION OF MICROBES A BY SCRATCHING

MATERIALS
Media
Luria Agar (LA) medium
Equipments
Ose needle, Bunsen lamp, Petri dish, pen
PROCEDURE
1. One bacterial isolate grown from culturing activities was transferred aseptically to a Petri dish containing Luria Agar (LA) medium by 4 quadrant method (Figure 1).
2. The first is done by dividing 4 parts of LA media inside a Petri dish as shown using a pen (1, 2, 3, and 4).
3. One colony is taken using an ose needle and scratched into the 1st area.
4. Furthermore, the ose needle is burned using Bunsen and scratched from area 1 to 2 and further scratching is carried out on area 2 without hitting area 1.
5. This is done successively until the scratching on area 4. An indicator of the success of this scratching process is that a single colony of bacteria is obtained

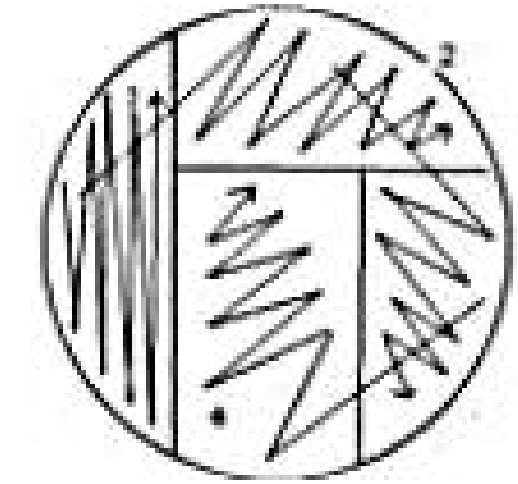


Figure 2. Isolate purification by the 4 quadrant method

MEETING 4. MORPHOLOGY OF BACTERIA

PURPOSE

1. Students study the morphology of bacteria by staining using one type of paint.
2. Students see the form and activity of live bacteria
3. Students recognize the forms of bacterial colonies in various media.

PRINCIPLE

Microbial colonies show different forms of both types and types. Fungi (thread fungi), yeast, bacteria, and actinomycetes have different colony growth rate characteristics, both medium flat colony growth (petri dish), upright or oblique. The various microbes above, bacteria that have a colony/morphological shape vary, both colony shape, elevation shape, edges and structure in bacterial colonies.

Bacterial cells are no color, so it is difficult to observe directly. To facilitate the observation of bacterial morphology, cell staining is necessary. The process of staining bacterial cells is usually called staining. The basis of staining is to color the background or certain parts of the cells so that the cells or their parts can be seen with more clarity/contrast.

1. Negative staining

Staining is carried out to color the background of the preparation and the bacteria themselves are not stained. In staining is not carried out fixation therefore it can be used to see the actual shape of the cell and to determine the size of microbes (bacteria)

2. Simple staining

Staining is carried out using one type of paint, before dyeing, bacterial cells are repaired first. Widely used paints are methylene blue (MB), gentiana violet, basic fuchsin or safranin.

3. Ziell Neelsen staining (quickly souring)

Ziell Neelsen's stainings are differential paints that use several types of substances, namely lar. ZN-A; ZN-B; and ZN-C. ZN dyes are intended for microbes that are difficult to color with gram stainings, since their cells contain a lot of fat or wax such as the genus *Mycobacterium* (*M. tuberculosis, M. leprae*) and some other actinomycetes. ZN-A contains red carbolic fuchsin (CF) where CF is more easily soluble in phenols and phenols can be soluble in fat or wax. Microbes containing a lot of fat and wax easily absorb phenol-soluble alkaline fuchsin.

4. Gram staining

Gram stainings are defensive stainings that use a variety of paints. Differences in the cell structure of Gram-positive (+) and Gram-negative (-) bacteria result in differences in their staining properties. The cell wall and cytoplasmic membrane of Gram-positive (+) bacteria have a large affinity for the purple crystal paint complex and iodine, while the Gram-negative (-) affinity is very small. The striking difference is that the cell wall of Gram-positive (+) bacteria consists of 90% peptioglycan layer while in Gram-negative bacteria (-) only 5-20%. In stainings with purple crystal paint (gram A) Gram-positive bacteria (+) will bind strongly to the paint and not fade with soft paint, as a result of which the color of the cells becomes blue. On the contrary, gram-negative bacteria (-) cannot bind strongly to purple crystal paint and fade with softening paint, as a result of which the bacterial cells are stained with the last paint, that is, sapphire, so that the cells are red.

5. Observation of bacteria with hanging preparations (Hanging drop)

With a hanging drop, bacterial cell preparations can be seen alive. The shape of the cell

can be transparently visible, as well as the active movement of bacteria can be observed in a lively way.

OBSERVATIONS OF MICROCOPIC

A. Simple staining

MATERIALS
Culltures
Pure culture *B. subtilis*; *E. coli at* NA age 24 hours
Reagents
Ziehl Neelsen paint solution (Fuchin carbol) or Hucker paint solution (crystalline purple)
Equipments
Object glass

PROCEDURE
1. Make preparations for the spread of bacteria
2. Drip the paint solution over the spreading preparation with 1-2 drops leave for 1-2 minutes
3. Wash under running water until the remaining paint is washed away, then dry it carefully using suction paper
4. Observe under a microscope using a magnification of 1000x using immersion oil
5. Draw and record the morphology of the observed bacteria

B. Observation of bacteria with hanging drops

MATERIALS
Culltures
Pure culture of *B. subtilis* in 24-hour-old NA media
Equipments
Notched object glass, cover glass and vaseline

PROCEDURE
1. Clean the glass of the notched object with alcohol and heat it over the flame of the Bunsen lamp. Smear the edges of glass basin objects with vaseline
2. Clean the cover glass from dust, then take 1 ose *B. subtilis* aseptically and place it on top of the cover glass.
3. The cover glass that has contained the bacterial suspension is quickly turned over so that the part where the suspension is located is below, and placed on the glass of the object above the concave part
4. The observation picture is the shape and also observes the movement of bacteria.

MACROSCOPIC OBSERVATIONS

MATERIALS
Culltures
Pure cultures of *B. subtilis, E. coli* and *Bacillus subtilis* in a Nutrient Medium up to 24 hours of oblique age:

Media

Upright Nutrient Agar media, nutrient slant agar media to be oblique and liquid nutrient media,

Equipments

Sterile Petri dish

PROCEDURE

1. Ways of inoculation

a. The upright nutrient media becomes and the upright agar nutrient medium is inoculated aseptically with pure bacterial culture using an inoculation needle by piercing into the tube

b. The nutrient medium for obliqueness is inoculated aseptically with pure bacterial culture using an inoculation needle in a straight stroke

c. Aseptically inoculated liquid nutrient media with pure bacterial culture using oceans

d. In the inoculation of bacteria by sprinkling, agar of the nutrient medium that has been thawed and desired to a temperature of ± 50ºC is removed by pure aseptical bacterial culture, then the tube is pushed and poured into a petri dish aseptically.

2. How to incubate

Culture is incubated at a temperature of 37ºC or a room for 48 hours or more. Culture on gelatin media is incubated at a temperature of 20ºC (since gelatin melts at a temperature of 22 ºC).

3. Ways of observation

a. The Upright Agar Nutrient Media

Drawings and annotations:

- Growth: even or uneven growth either on the surface or the bottom of the substrate. Forms of growth in the first
- puncture : filliform, echinulate, bead, villous, rhizoid, arborescent

b. The Slant Agar Nutrient Media

Draw and annotate:

- Growth: thin, medium, bushy, or absent
- Forms of growth at puncture marks: filliform, echinulate, beaded, spreading, rhizoid, arborescent, plumose
- Elevation: flat, effuse, raise, convex
- Lightning (shine): glossy lime, non-glossy
- Topography: slippery, irregular, the surface is corrugated, contoured,
- wrinkles, verrucose
- Color (chromogenesis): red, yellow, green, brown, fluorescent.
- Optical features: opaqua, translucent, opalescent, colorful.
- Smell: odorless, smells like.....
- Consistency: slimy, grainy, boutique, viscid, brittle membrane.
- Medium color: to gray, brown, red, blue, green, no discoloration occurs

c. Upright Gelatin Nutrient Media

Draw and annotate:

- Growth: evenly or unevenly distributed; its growth occurs either on the surface or the base
- Forms of growth at puncture marks: filliform, echinulate, beads, papilate, villous, arborescent, plumose
- Gelatin liquefaction form: crateriform, resistance, infundibutiform, samiate, stratiform,

no thawing occurs, slow thawing, thawing fast.

- Medium color: fluorosense, becomes brown, no changes occur

d. Liquid nutrient medium

Drawings and annotations:

- Growths on the surface: ring, pellicie, flocullent, membrane, do not form a membrane.
- Turbidity: slight, medium, great
- Smell: odorless, smells like .....
- Precipitate: compact , uncertain in shape and size, granular (grain), multi-layered (scaly), viscous, numerous, few, no precipitate

e. Mediun nutrition for sprinkling

Draw and annotate:

- Growth: the growth of colonies on the surface or below the surface of the medium
- Colony forms: punctiform, circuler, filamenteus, irregular, curled, amoeboid, myceloid, rhizoid
- Surface: slippery, rough, forming concentric circles, like a light comb (radiating)
- Height: flat, effuse, raised, convex, umbonate
- Edge shape: whole, wavy, lobate, erose, filamentous, curved.
- Forms of the inner structure: amorphous, finely grained or gauze, filament-like, curved, concentric

f. Medium sprinkling of gelatin

The observation is the same as on the nutrient medium so that the sprinkling above with the addition of gelatin melts: gelatin thawing: cups, plates, evenly distributed

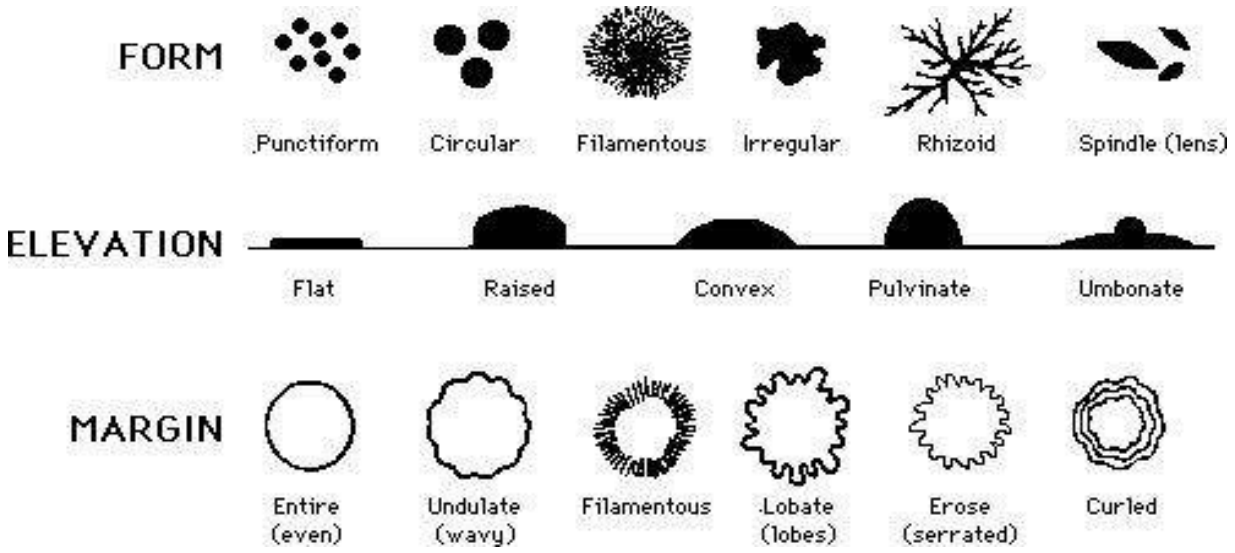


Figure 3. Morphology of bacterial colonies

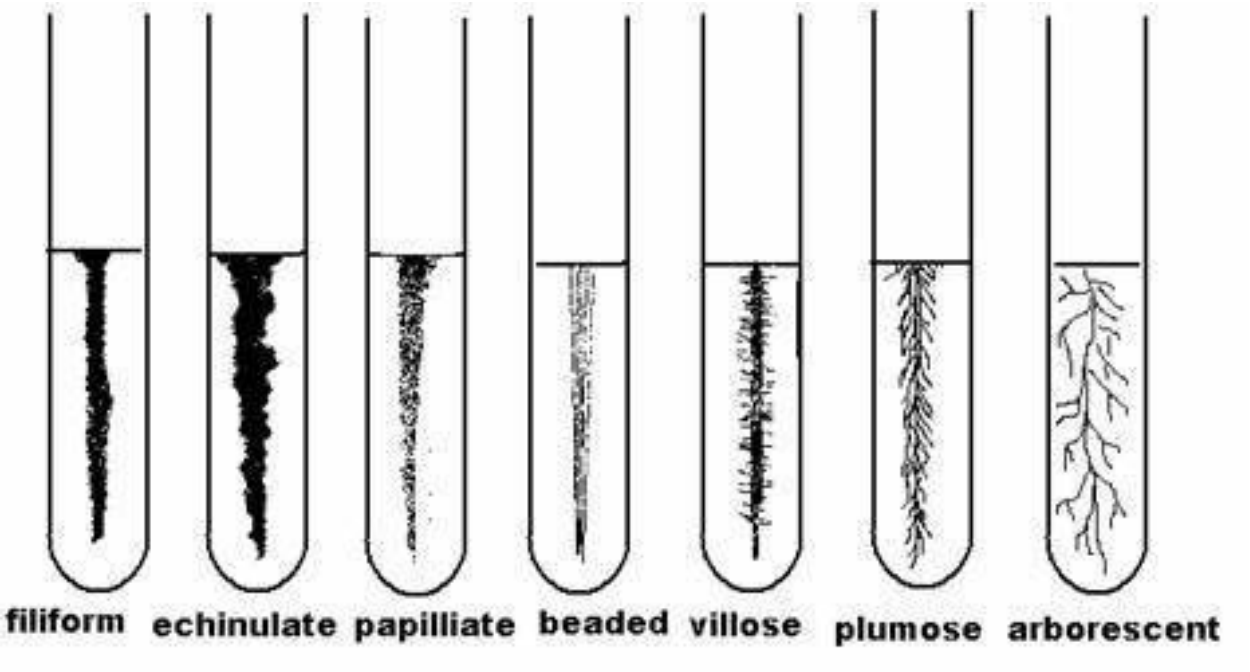


Figure 4. The growth of colonies on the upright medium

MEETING 5. MORPHOLOGY OF ACTINOMYCETES

PURPOSE

1. Students recognize the morphology of various genera of actinomycetes
2. Students study the microscopic morphology of actinomycetes cells

PRINCIPLE

Actinomycetes are a group of unicellular mycelium organisms, whose way of reproduction is through division or through spores or special conidia. These microorganisms are closely related to true bacteria; often thought to have higher taxonomic levels, as filamentous bacteria. During their growth, these microorganisms form substrate mycelium (vegetative) only or two types, namely substrate mycelium (vegetative) and aereal/air (as the part that forms spores/sporogen).

At the beginning of the description, actinomycetes are often defined as unicellular microorganisms, 1μm in diameter, forming filaments, monopodial branching, rarely dichotomous, resulting in colonies with creeping structures. Its two commonly known forms of reproduction are: (a) fragmentation, or the formation of conidia, and (b) segmentation. Both types of spores grow both in the medium and form branched mycelia.

Actinomycetes are commonly known as a large heterogeneous group of microorganisms, consisting of several genera and a large number of species. Actinomycetes have great variations in morphology, physiology, biochemical activity, and their role in natural processes.

The morphology of actinomycetes can be easily observed using slide cultures incubated in a damp room. The attachment of the mycelium to the glass cover of the object placed cornered on the growing culture, can be transferred to the glass of the object and observed with high magnification.

Morphological characters are still widely used for the characterization of genera, for example the presence or absence of spores in the substrate mycelium or the zoospores formation of special spore vesicles or spore boxes. The morphological picture of actinomycetes includes the presence of mycelium, conidia, sporangia and other structures.

1. Mycelium

It is stable or temporary, if it is damaged it should be observed its parts and their motility (*Oerskovia* sp. releases the element). The mycelium formed is the substrate mycelium and the air is formed, or just the substrate mycelium alone (very common), or just the air hyphae alone (very rarely-Sporichthya). Mycelium can form inserted vesicles that do not contain spores (Intrasporangium) or contain many spores (Frank).

2. Conidia

The term is used for asexual spores that are not intercaler chlamydospores or sporangiospores. Actinomycetes form conidia in different ways :

a. One conidia found in several genera

The genus *Thermoactinomycetes* (Group 28), is famous for its heat-resistant endospores. Nonthermostabil single conidia are found in the genera *Saccharomonospora* and *Promicrospora* (Group 22). *Micromonospora* (Group 24) and *Thermomonospora* (Group 27). Meanwhile, members of the Frankish genera, *Dactylosporangium*, and Intrasporangium sometimes form end vesicles confused with spores. Also some other organisms such as actinomadurae, will form a single vesicle when grown under unsuitable conditions.

b. Conidia pairing

Longitudinal conidia pairs are characters of the genus *Microbispora* (Group 26) which is formed only in the air mycelium.

c. Conidia short chain

Although it is difficult to determine how long the short chain of conidia is, conidia chains of up to 20 spores are usually considered short. Representatives of the following genera form conidia chains: *Nocardia*, *Psedonocardia*, and *Saccaromonospora* (Group 22); *Streptoverticillium* and *Spirochtya* (Group 25), *Actinomadura* and *Microtetraspora* (Group 26); *Streptoallotechus* (Group 27); and *Glycomyces* (Group 29). This type of morphology can also be grouped into the genera *Amycolata* and *Amycolatopsis* (Group 22) and *Catellatospora* (Group 24). Some Microellobosporia-type Streptomycetes form a short chain, which is surrounded by a sheath and can be observed using a light microscope.

d. Long chain of conidia

Some genera of Actinomycetes have a long chain of conidia, namely *Nocardia*, *Nocardiales*, *Psedonocardia, Saccharoplolyspora, Actimopolyspora,* and *Amycolatopsis* (Group 22)*; Strepttomyces* and *Streptoverticillium* (Group 25); *Actinosynnema*, *Nocardiopsis, Streptoallotechus* (Group 27); and *Kibdelsporangium*, *Kitasatosporia*, *Glycomyces*, *Saccharothrix* (Group 29).

e. Conidia in hyphae coalesce into motile spores that liberate the cinemata (Actinosynnema, Group 27).

3. Sporangia

Sporangia is a spore sac. The bag-shaped structure of sporangia is where spores develop and are bound together until the release of spores occurs, so that the sporangia is empty and is a membrane. The membrane is not integrated into the spore part. Sporangia originates in: (a) on well-developed aerial hyphae or on the surface of colonies with little or no air hyphae (*Actinoplanes*, *Ampullariella*, *Pilimelia*, *Dactylosporangium* (Group 24); *Planobispora*, *Palnomonospora*, *Spirillospora*, *Streptosporangium* (Group 26); or (b) especially in AGAR media (*Kineospora*, Group 25).

4. Structure lainnya

Some actinomycetes form an unusual structure. Spores in synemata are found in Genus *Actosynnema*. These organisms in Group 23 form spore masses resulting from the division of several planes, not from the perpendicular division of the hyphae axis. The structure of spore laying is called multilocular sporangia.

Some actinomycetes will form spherical structures in the air hyphae. These are not water droplets that condense like curved spore chains, or their structure may contain hyphae embedded in the amorphous matrix (*Kibdelosporangium*, Group 29).

Sclerotia is a globose treated by some strepotomycetes. Skelotia contains no spores but lipid-containing cells. It germinates completely, as pseudosporangia from kibdelosporangia. The air morphology and surface growth of some genera in groups 22-29 are shown schematically in the figure below. Morphology is useful for the identification of some genera but not for all of them.

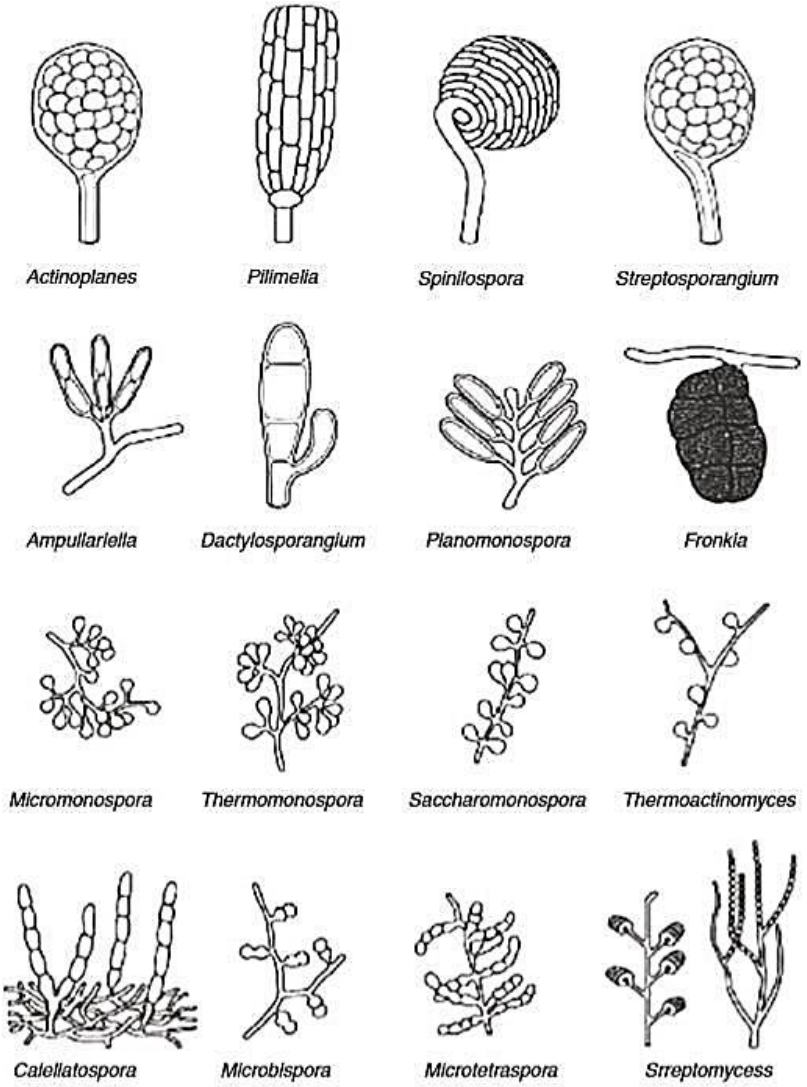


Figure 5. Morphology of actinomycetes spores

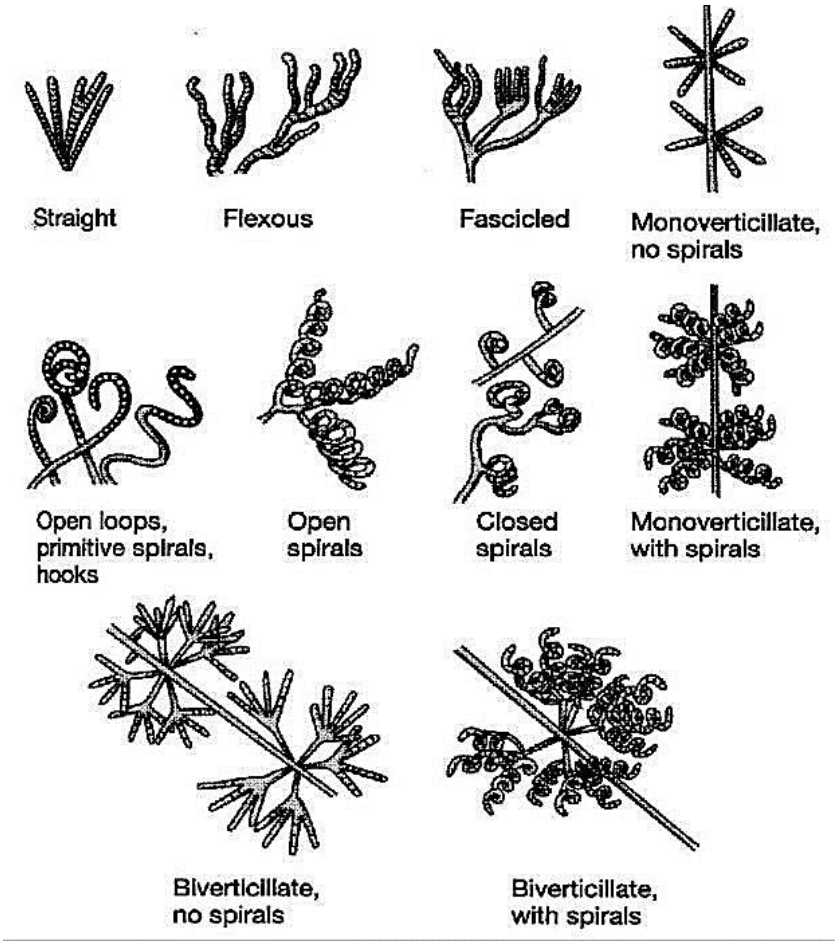


Figure 6. Structure of *Streptomyces* spora

MACROSCOPIC OBSERVATIONS

MATERIALS

Cultures

Cultures containing actinomycetes

Media

Agar Extract Malt Yeast Media (YMA), YMA media is tilted on the test tube

Equipments

Sterile Petri dish, Magnifying glass

PROCEDURE

1. Melt the upright substrate in a water bath and pour it aseptically into sterile dishes, wait until it cools and solid
2. Inoculation of a slight culture of actinomycetes on two plates and one inclined tube to make YMA
3. Incubation of both, cups upside down at room temperature for 2 to 7 days or more.
4. Observe colony changes that indicate the color properties of the colony and pigmentation and colony shape that include the appearance of a dense, rough, cone-shaped , dry surface, often

enveloped by air mycelium and observe the presence of spores

5. Actinomycetes that produce two types of mycelium namely the substrate or vegetative mycelium and air usually form special reproductive cells, known as spores or conidia

MICROSCOPIC OBSERVATIONS

MATERIALS

Cultures

Culture of actinomycetes

Media

YMA media

Equipments

Sterile drains, sterile Petri dish, object glass, sterile cover glass, sterile toothpicks, microscope, Inoculation needle, and Bunsen Lamp

PROCEDURE

1. Glass culture of objects

a. A thin tuft of agar is cut from the allocated Petri dish sequentially, placed on the glass of the sterile microscope object then inoculation and cover with sterile cover glass.

b. After incubation in a damp room, observe the glass culture of the object directly with a microscope, observe the air mycelium and the substrate in order.

2. Glass cover of an inclined object for morphological observation of actinomycetes

a. Inoculation of a AGAR pan with a slight culture of actinomycetes and tucking the glass cover of the object at an oblique angle near the scratch.

b. After incubation, take the object glass cover and attach it to the glass of the object, the upper surface at the bottom, dripping water.

MEETING 6. MORPHOLOGY OF FUNGI AND YEAST

PURPOSE

1. Students recognize the morphological characteristics microscopically in order to distinguish species from each other
2. Students study the morphology of the colony, the character of the growth of each type of fungus on the medium
3. Students study the different forms of yeast cells and distinguish between dead and living cells
4. Students dye the cell spore, look at the different forms of yeast spores and count the number of spores in the cell

PRINCIPLE

Fungal Morphology

To find out the name of the genus and species of fungal culture is not so easy, so the identification stage is required. The first stage for carrying out identification is the introduction of morphological features both microscopic and their macroscopic morphology. The color of the colony, the surface of the colony, the presence or absence of radial lines or concentric circles (zoning) and the presence or absence of liquid points (exudate drops) above the surface of the colony are characters that need to be considered and considered in the macroscopic identification of the colony. In addition, the shape and size of sporangiums, sporangiospores, mycelium branching forms, branching and conidial heads are often the subject of accurate consideration for determining their species. A beginner can be sure of the difficulty of distinguishing the Genus *Penicillium* and the Genus *Paecilomyces*. However, by looking at the macroscopic development of the colony and the microscopic properties of the conidial head, the two genera can be clearly distinguished.

Yeast morphology

Yeast or yeast is a fungus that is single-celled and does not form mycelium, however, some species of which can form pseudo-mycelium. The morphology of yeast is simpler than that of fungi, however, it is larger in size than that of bacteria. Important morphological properties of yeast include: the shape of the cell (oval, round like a sausage, ellipse), cell size, shape and number of buds, shape as well as the number of spores inside the hermit, shape/formation and the presence or absence of pseudo-mycelium are essential for identification.

MICROSCOPIC OBSERVATIONS OF THE FUNGI MORPHOLOGY

MATERIALS

Cultures

Pure culture of *R. oryzae*; *A. oryzae*; *Penicillium* sp

Reagent

Solution of lactophenol or lactophenol blue, Alcohol 70% and Bunsen ethyl alcohol

Equipment

Object glass, cover glass, cotton, ose needle

PROCEDURE

1. Clean the glass of the object and cover the glass with 70% alcohol until it is fat-free, then drip a few drops of lactophenol solution or a cotton swab of blue lactophenol on the glass surface of the object.

2. Take a small number of culture colonies with an inoculation needle, place them in lactophenol droplets and carefully decomposition them with a needle of the preparation. Keep the entire mycelium wet exposed to lactophenol.
3. Cover with a glass cover in such a way that there are no air coils in the preparation. Clean excess lactophenol with suction paper.
4. Observe with a microscope using an objective lens of 10x magnification then with a magnification of 40x. To see the morphology of conidia or spores use an objective lens of 100x magnification with immersion oil.
5. Record and draw all observed such as branched or unbranched mycelium, bifurcated or not, smooth or rough, sterigma, conidia, spores, conidiophores, spores, sporangiophores, columella, vesicles, etc.

MACROSCOPIC OBSERVATIONS OF THE FUNGI MORPHOLOGY

MATERIALS
Cultures
The pure culture of *Aspergillus*; *Rhizopus; Penicillium*; and *Mucor*
Reagents
Alcohol 70%
Media
Media PDA, CDA
Equipments
Sterile petri dishes and inoculation needles and Bunsen lamps

PROCEDURE
1. Heat the prepared upright PDA/CDA media until it melts aseptically into a sterile petri dish, wait for it to cool and harden.
2. Slightly remove the extraneous culture of each species of fungus on the surface of the substrate
3. Incubation for 4-5 days at room temperature
4. Observe daily the discoloration that appears in the colony, the state of the confluence of the colony (flat, flour-like mountains, coarse or cotton-like), the presence or absence of radial stripes, the presence or absence of concentric lines or circles (zoning), the presence or absence of exudate droplets and their color, the presence or absence of a characteristic odor and the shape and color of the colony.
5. Record everything that is observed

MORPHOLOGY OF THE YEAST

YEAST CELL DYEING

MATERIALS
Cultures
Pure culture of *Saccharomyces cerevisiae*; *Candida* sp.; *Hansenulla* sp. in 72 medium hours
Reagents
Methylene blue solutions, alcohol and Bunsen ethyl alcohol
Equipments
Object glass, cover glass, ose needle

PROCEDURE

1. Clean the glass of the object using 70% alcohol until fat-free
2. Drop a little MB over the glass of the object.
3. Take with a small ose needle a yeast culture, put it in the mb droplets and cover it with a covering glass, trying not to allow air bubbles.
4. Observe under a microscope using a magnification of 40x and 100x using immersion oil.
5. Drawing and recording : cell shape; the presence or absence of buds, the number of buds in each cell, the presence or absence of pseudo-mycelium (pseudo-mycelium).

YEAST SPORE STAINING

MATERIALS

Cultures

Pure culture of *S. cerevisiae*; *Candida* sp.; *Hansenulla* sp. age 72 hours or more on medium agar carrot (carrot slant agar) or carrot sliced medium (carrot slices)

Reagents

Aniline crystal violet paint solution (paint A); safranin paint solution (paint B); a solution of acidic alcohol and a glass of object.

Media

A carrot slant agar, carrot sliced medium

Equipments

a glass of an object, Bunsen lamp, microscope

PROCEDURE

1. Suspend the pure culture of yeast in a sterile aqueduct, then take one ose flattened on a glass of an object about 1 cm2.
2. Dry wind, glued together with a Bunsen lamp 5-7 times over the fire.
3. Drip the culture stains with paint A and heat them for 3 minutes, so as not to dry out.
4. Wash under running water,
5. Fade with acidic alcohols.
6. Wash under running water
7. Drip with paint B for 10-15 seconds (to be precise)
8. Wash under running water and dry wind
9. Observe with a microscope on a strong magnification with emersion oil
10. Draw and give a full description of yeast cells and their spores.

MEETING 7. MORPHOLOGY OF ALGAE

PURPOSE

Students can find out the types of microalgae found in fresh water, rice field water, and pond water.

PRINCIPLE

Algae are talus plants that live in water, both freshwater and seawater. Types of algae that live freely in water, especially those whose body is single-celled and can move actively are phytoplankton constituents. The actively movable type has a device for movements that whip feathers or flagella. Flagella are more than one in number and when their length is the same isocon, heterocon when the length is not the same. In addition, in algae, spores and gametes can usually move actively with flagella intermediaries as well. Spores that can move actively are called zoospores.

Although the body of algae shows a very large degree of diversity, but all its cells always clearly have nuclei and plastids, and in their plastids there are chlorophyll-derifate dyestuffs, chlorophyll-a or chlorophyll-b, or both. In addition to chlorophyll derifate, there are also other dyestuffs that often stand out more and cause certain groups of algae, so they are named after these colors. These coloring agents are phycocyanin (blue dye), phycosantin (golden yellow), phycoerythrin (red). In addition, it is also common to find coloring agents santofil and carotene (Tjitrosoepomo, 1989).

The division of algae divisors is distinguished mainly over: (1) the chemical properties of their photosynthetic pigments include chlorophyll and additional pigments; (2) chemical regulation of its food reserves; (3) the number and type of flagella in each cell that moves in its life cycle. Additional properties such as the chemical content and structure of cell walls, the type of breeding, and the morphology of breeding tools are also used in the classification, especially in individual divisions (Loveless, 1989).

MATERIALS

Cultures

Fresh water, pool water, and rice field water

Reagent

Ringers solution

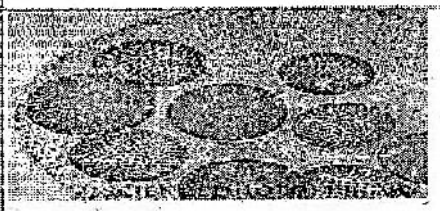
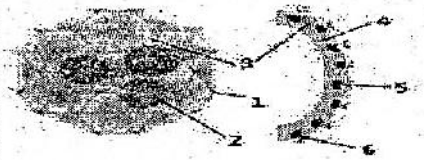
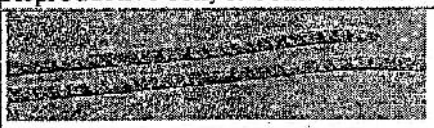
Equipment

Microscope, object glass dan cover glass, cotton dan needle, Scalpel and surgical tub

PROCEDURE:

1. Take samples (fresh water, pool water, and rice field water) using a drip pipette then drip on the cover glass and cover with a cover glass, lest there be bubbles.
2. Observed the presence of algae in the sample using a microscope with the smallest magnification.
3. Then draw the results of these observations.

OBSERVATION

Gambar	Gambar Rujukan	Taksonomi
Volvox	  *Volvox* : 1. *Chlamydomonas*-like cell, 2. Dughter colony, 3. Cytoplasmic bridges, 4. Intercellular gel, 5. Reproductive cell, 6. Somatic cell.	Divisio : Chlorophyta Kelas : Chlorophyceae Ordo : Volvocales Familia : Volvocaceae Genus : Volvox Spesies : *Volvox sp.*
Oscillatoria sp.	 	Divisio : Chyanophyta Kelas : Chyanophyceae Ordo : Oscillatoriales Famili : Oscillatoriaceae Genus : Oscillatoria Spesies: *Oscillatoria sp*

I. ALGAE

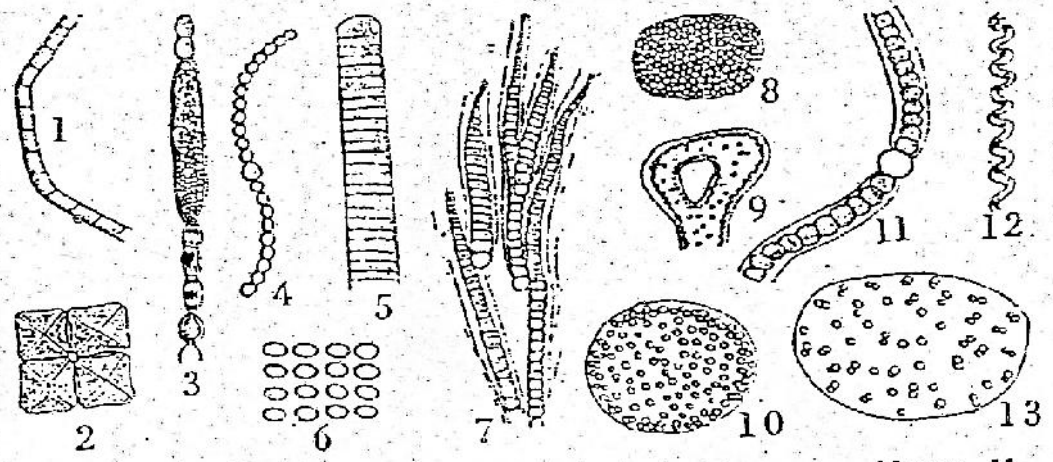
Neither all algae nor all protozoans may be clearly divided simply into plants and animals. Too much overlap occurs in habits, structures, and their physiology. For this reason, some flagellated forms such as *Chlamydomonas*, *Volvox*, and *Eudorina* are included here both as algae and protozoans. For a discussion of this problem, see Stanier in Ward and Whipple, *Fresh-water Biology*, 1959, pp. 7-15.

KEY I: ALGAE

			Plate ref.
1	–Cells blue-green; pigments not in chloroplasts. The blue-greens: MYXOPHYCEAE	*2*	IA
	–Cells green, red, or brown	*18*	
2	–Cells unicellular or in clusters and colonies, never with cells in filaments; commonly embedded in a gelatinous matrix, more rarely freely floating: Chroococcales	*3*	
	–*Cells* (except Spirulina, IA-12) filamentous; branched or unbranched, multiplication by filamentous active hormogones: Hormogonales	*9*	
3	–Cells solitary or in colonies of less than 50 cells:		
	–Cells spherical	*Chroococcus*	
	–Cells cylindrical	*Synechococcus, Chroothece*	
	–Cells fusiform	*Dactylococcopsis*	
	–Numerous cells in gelatinous matrix	*4*	
4	–Colonies and gelatine without definite form	*5*	
	–Cells in definite arrangement	*6*	
5	–Cells in several gelatinous capsules:		
	–Cells spherical enclosed in shapless masses of gelatine	*Gloeocapsa*	
	–Cells elongate or elliptical	*Gloeotheca*	
	–Cells scattered within the gelatinous matrix:		
	–Cells spherical	*Aphanocapsa*	IA-13
	–Cells elongate	*Aphanothece*	
6	–Colonies free floating	*7*	
	–Colonies attached, epiphytes on algae	*Chamaesiphon*	
7	–Cell division in 3 planes, colonies therefore in clumps:		
	–Cells spherical; colonies spherical when young, torn and net-like when older	*Polycystis (Mycrocystis)*	IA-8, 9
	–Cells wedge-shaped, colonies spherical	*Gomphosphaeria*	
	–Cell division in 2 planes, colonies therefore only one cell deep	*8*	
8	–Colonies a hollow sphere without radiating strands	*Coelosphaerium*	IA-10
	–Colonies plate-like with rounded cells	*Merismopedia*	IA-6
9	–Filaments not attenuated and hair-like at ends	*10*	
	–Filaments conspicuously attenuated towards one or both ends: RIVULARIACEAE. Filaments each with a basal heterocyst; filaments radiating in a gelatinous mass	*Rivularia, Gloeotrichia* (et al)	IA-7
10	–Filaments usually not branching	*11*	
	–Filaments branching:		
	–True branching	STIGONEMACEAE	
	–False branching, heterocysts present	SCYTONEMATACEAE	

11	–Cells of the filaments all of uniform size, without heterocysts: OSCILLATORIACEAE		*12*
	–Filaments with occasional cells of different color or larger size (heterocysts): NOSTOCACEAE.		*16*
12	–Filaments without sheath		*13*
	–Filaments enclosed in gelatinous sheath		*14*
13	–A single spiral cell	*Spirulina*	IA-12
	–Multicellular:	*Arthrospira*	
	–Filament a spiral	*Oscillatoria*	IA-5
	–Filament not a spiral		*15*
14	–One filament within a sheath	*Schizothrix, Microcoleus*	
	–More than one filament within a thick sheath		
15	–Sheath slimy, filaments often bent, agglutinated	*Phormidium*	IA-1
	–Sheath firm, not slimy; filaments not in bundles	*Lynbya, Symploca*	
16	–Filaments contorted, within a definite gelatinous sheath	*Nostoc*	IA-11
	–Filament more or less straight, free or in formless slimy mass, without sheath		*17*
17	–Heterocysts terminal; spores long and cylindrical	*Cylindrospermum*	
	–Heterocysts not terminal:		
	–Filaments aggregated without order	*Anabaena*	IA-4
	–Filaments in bundles of plate-like masses	*Aphanizomen*	IA-3
18	–Organism green, not yellowish green; if reddish then unicellular		*19*
	–Organism yellowish green, red, or brown		*48*
19	–Organism with whorles of leaves: CHARACEAE	*Chara, Nitella*	
	–Organism smaller, without whorls: CHLOROPHYCEAE		*20*

PLATE IA: BLUE-GREEN ALGAE



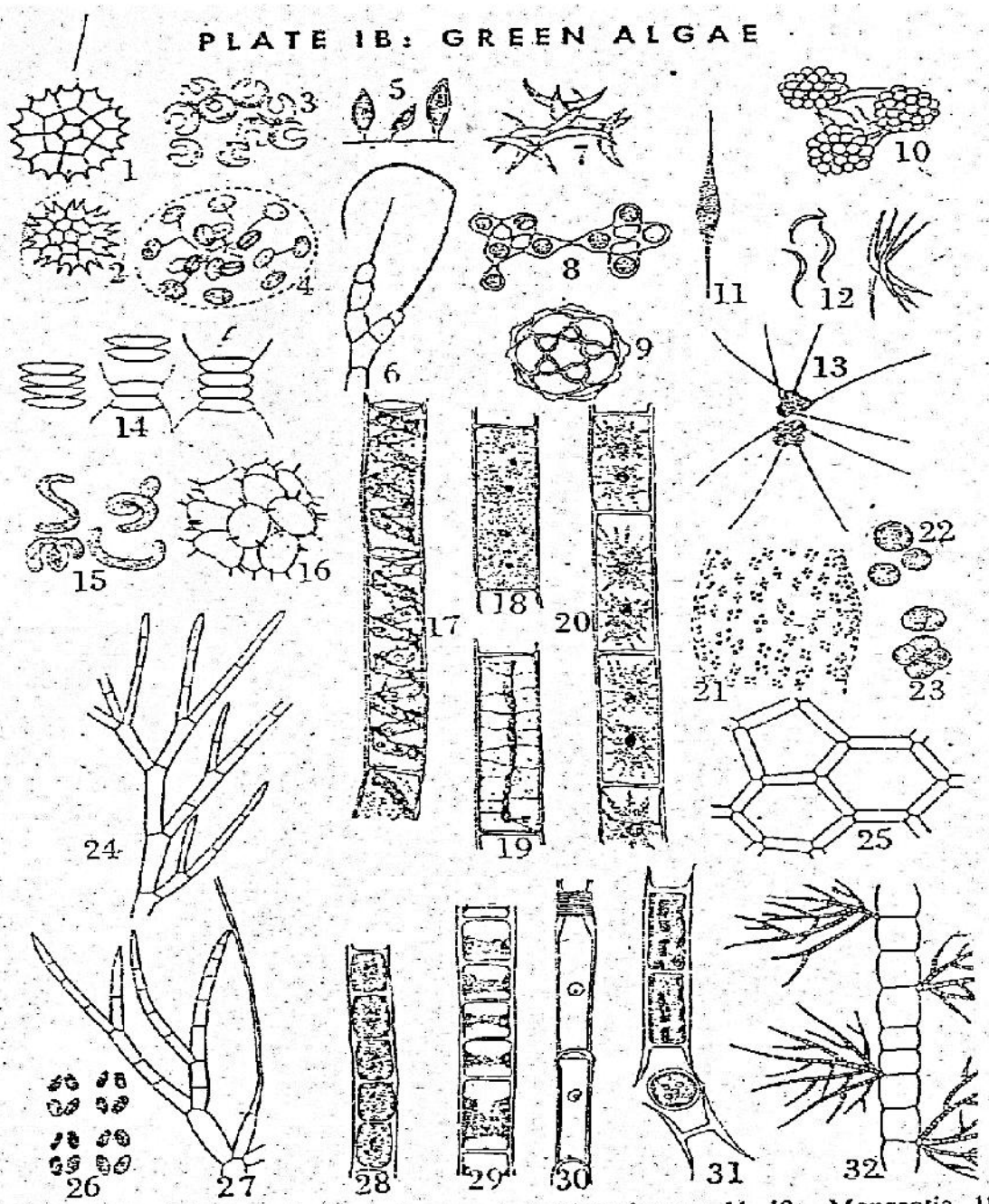
1. Phormidium
2. Tetrapedia
3. Aphanizomenon
4. Anabaena
5. Oscillatoria
6. Merismopedia
7. Rivularia
8, 9. Polycystis
10. Coelosphaerium
11. Nostoc
12. Spirulina
13. Aphanocapsa

Anabaena, 4
Aphanocapsa, 13
Aphanizomen, 3
Coelosphaerium, 10
Merismopedia, 6
Polycystis, 8, 9

Nostoc, 11
Oscillatoria, 5
Phormidium, 1
Rivularia, 7
Spirulina, 12
Tetrapedia, 2

20	–Thallus expanded, membranous	*Ulvales*	
	–Thallus neither expanded nor membranous	*21*	
21	–Thallus not filamentous; no conjugation	*22*	
	–Thallus filamentous, though filaments may unite in a plane; if unicellular, conjugation takes place	*39*	
22	–Unicellular or of a definite number of flagellated, motile cells	*23*	IIA, IIB
	–Cells not flagellated or motile (see figs.)	*27*	IIA, IIB
23	–Composed of colonies of many cells with two flagella	*24*	
	–Composed of single cells with 2 or rarely 4 flagella:		
	–Contents of cell close to cell wall	*Chlamydomonas*	IIA-3
	–Contents of cell connected to cell wall by threads	*Haematococcus*	
24	–Colonies spherical or circular	*25*	
	–Colonies flat, cells 4-16 or 32, angles rounded in a colorless sheath	*Gonium*	
25	–No gelatinous cover:		
	–Many cells in a hollow globe	*Volvox*	IIA-21
	–Cells 8-16, arranged in 4 tiers	*Spondylomorum*	
	–With a gelatinous cover	*26*	
26	–Colonies round or spherical		
	–Cells 16-32-64, globose, not crowded	*Eudorina*	IIA-7, 8
	–Cells 4, 8, 16, 32, globose, crowded	*Pandorina*	IIA-13
	–Colony of 8 cells in an equatorial zone in a spherical or ellipsoidal covering	*Stephanosphaera*	
27	–Cells formed in plates or network: HYDRODICTYACEAE		
	–Cells in a flat plate	*Pediastrum*	IB-1, 2
	–Cells form a network	*Hydrodictyon*	IB-25
	–Cells not in a plate or network	*28*	
28	–Unicellular and solitary; cell with differentiation of base and apex: CHARACIACEAE	*Characium*	IB-5
	–Cells without differentiation of base and apex	*29*	
29	–Unicellular and globular or consisting of short, few celled filaments; firm cell wall. Often in damp situations. PLEUROCOCCACEAE	*Protococcus*	IB-22, 23
	–Not as above	*30*	
30	–Cells spherical and indefinite in number, embedded in a copious gelatinous envelope	PALMELLACEAE, TETRASPORACEAE	IB-21
	–Colonies free or colonial without copious gelatinous envelope; forming autospores	*31*	
31	–Cells elongated, frequently curved; solitary or in definite loosely coherent colonies	*32*	
	–Cells not elongated	*34*	
32	–Colonies enveloped in mucus	*Kirchneriella*	IB-3
	–Colonies with little or no mucus	*33*	
33	–Cells attenuated to acute spines:		
	–Cells forming definite colonies each of a single row	*Scenedesmus*	
	–Cells solitary or loosely grouped in irregular bundles	*Ankistrodesmus*	IB-11, 12
	–Cells lunate, arranged back to back	*Selanastrum*	IB-7
	–Cells sublunate or ellipsoidal, arranged in groups forming irregular colonies	*Dimorphococcus*	
34	–Cells variable, united in a regular flat plate	*Crucigenia*	IB-26
	–Cells not united in a flat plate	*35*	
35	–Cells angular with a definite number of angles; cells solitary	*Tetraedron*	
	–Cells globose or subglobose	*36*	

PLATE IB: GREEN ALGAE



1, 2. Pediastrum
3. Kirchneriella
4. Dictyosphaerium
5. Characium
6. Bulbochaete
7. Selenastrum
8, 9. Coelastrum
10. Botryococcus
11, 12. Ankistrodesmus
13. Richterella
14. Scenedesmus
15. Ophiocytium
16. Sorastrum
17. Spirogyra
18, 19. Mougeotia
20. Zygnema
21. Tetraspora
22, 23. Protococcus
24. Cladophora
25. Hydrodictyon
26. Crucigenia
27. Chaetophora
28. Microspora
29. Ulothrix
30. Oedogonium
31. Tribonema
32. Draparnaldia

Ankistrodesmus, 11, 12
Botryococcus, 10
Bulbochaete, 6
Characium, 5
Chaetophora, 27
Cladophora, 24
Coelastrum, 8, 9
Crucigenia, 26
Dictyosphaerium, 4
Draparnaldia, 32
Hydrodictyon, 25
Kirchneriella, 3
Microspora, 28
Mougeotia, 18, 19
Oedogonium, 30
Ophiocytium, 15
Protococcus, 22, 23
Pediastrum, 1, 2
Richterella, 13
Scenedesmus, 14
Selenastrum, 7
Sorastrum, 16
Spirogyra, 17
Tribonema, 31
Ulothrix, 29
Zygnema, 20

Cells strictly globose, united in a spherical colony:		
—Sphere hollow	*Coelastrum*	IB-8, 9
—Sphere solid, cells with stout spines	*Sorastrum*	IB-16
Cells not united in a spherical colony	*37*	
Cells with two or more attenuated bristles	*Micractinium*	
Cells without bristles	*38*	
Cells generally retained within large wall of mother cell	OÖCYSTACEAE	
Cells in grape-like clusters, freely exposed in a thin gelatinous envelope	*Botryococcus*	IB-10
Cells with well-marked subdichotomous connecting threads, chloroplast parietal	*Dictyosphaerium*	IB-4
Cell division by intercalation of new cells producing transverse striation.		
Oedogoniales:		
—Cells long, filaments unbranched	*Oedogonium*	IB-30
—Cells short with laterally placed bristle, filaments branched	*Bulbochaete*	IB-6
Cell division of the ordinary type	*40*	
Filaments attenuated and commonly ending in a tapered thread	*41*	
Filaments not ending in a tapered thread	*46*	
Plant of branched filaments forming a flat cushion-like expansion	*Chaetosphaeridium, Coleochaete*	
Plant entirely filamentous	*42*	
Filaments branched	*43*	
Filaments not branched	*45*	
Plants less than 1 cm. high, without setæ	*Microthamnion*	
Plants larger, branches attenuated, and with setæ	*44*	
Filaments fine, showing little difference in character of main axis and branch, not in tufts in gelatinous masses	*Stigeoclonium*	
—Filaments in tufts in a dense gelatinous mass	*Chaetophora*	IB-27
—Filaments and main branches large, bearing tufts of small branches	*Draparnaldia*	IB-32
Cells with thick lamellose coats, in a series inside a lamellos sheath	*Cylindrocapsa*	
Cells without lamellose coat:		
—Chromatophore a homogeneous zonate band, with one to several pyrenoids	*Ulothrix*	IB-29
—Chromatophore a parietal disk or plate, with one pyrenoid	*Stichococcus*	
—Chromatophore granular, covering more or less completely the whole cell wall, containing starch but no pyrenoids	*Microspora*	IB-28
Chloroplast a parietal reticulum with scattered pyrenoids or in form of numerous discs, some containing pyrenoids	*Cladophora*	IB-24
Chloroplasts single or several, large and of definite shape, with pyrenoids. The entire contents of two cells unite to form a single zygote.	Zygnematales	*47*
Thallus an unbranched thread of many similar cells; sexual reproduction by formation of conjugation tubes between cells of parallel filaments or adjoining cells of the same filament.		
—Chloroplasts spiral bands	*Spirogyra*	IB-17
—Chloroplasts of two stellate bodies for each cell	*Zygnema*	IB-20
—Chloroplast an axial plate	*Mougeotia*	IB-18, 19
Unicellular, rarely bound together in a loose thread.	DESMIDIACEAE (Desmids, Key IC)	IC
Organism yellowish green. Xanthophyceæ	*49*	
Organism grayish or brownish or amber colored	*50*	

MEETING 8. MORPHOLOGY OF PROTOZOA

PURPOSE

1. Recognize the diversity of protozoa
2. Observation of the morphology and structure of protozoa
3. Grouping protozoan animals into different classes based on similarities and feature differences

PRINCIPLE

Protozoa live freely in humid or watery places, however, there are also protozoa that live parasitically in the body of other organisms. These organisms breathe in a diffuse way. Protozoa can also form cysts to live longer in unprofitable situations. After the situation improves, the cyst will rupture and the protozoa will be active again.

The characteristics of protozoa include :

1. Ectoplasm (outer part) can produce cell walls in the form of pedicula (cell membranes) or testa (hard shells).
2. Endoplasm (inside). Place the cell organelles.
3. Food vacuoles (non-contractile vacuoles), which are fluid-filled cavities that surround food.
4. Contractile vacuoles (clinking of vacuoles), that is, organella that serve to expel metabolic remains and regulate pressure (osmoregulator)
5. In general protozoa have one eucarion (true nucleus) except Paramaecium which has 2 nuclei
6. Protozoa have a different way of eating. How to eat protozoa can be distinguished from:
   a. Parasite, attacking food in the form of body fluids of its host through the surface of its body
   b. Holozoic, a protozoa that feeds on other, smaller organisms such as bacteria, algae, or other protozoa.
   c. Holophytes, protozoa that have chloroplasts and are able to photosynthesize like greenery
   d. Saprophytes, prozoans that feed on organic matter from plant or animal remains

MATERIALS

Cultures

Hay culture, River water, pond water and paddy water, preparations for the preservation of Protozoa

Reagent

Ringers solution

Equipment

Microscope, Glass objects and glass covers, Cotton and needles, Scalpels and surgical tubs,

PROCEDURE

Hay culture

1. A total of 100 gr of straw is heated in 1 liter of well water until a soft straw
2. After that it is boiled ± 90 minutes then cool
3. Straw water is transferred to used bottles coupled with paddy water
4. Then left open for 7-10 days

Rhizopoda, Ciliata and Flagellata

1. The microscope and its fixtures are made in advance
2. Protozoan cultures of straw water baths, pond water, paddy water and river water are stored in the

preparation
3. Preparations are observed and visible animals are classified according to their respective classes

Sporozoa

1. The earthworms that have been brought are washed first
2. Then anesthetized with 70% alcohol in a goblet glass
3. The worm is dissected longitudinally from the anterior end to the final border of the clitoris (thickening), at the time of dissecting the worm is dripped with a ringing solution
4. The seminal vesicula, which takes the form of two pairs of milky white lumps/turns, is taken one of them and placed on a glass of a clean object that has been dripped by the ringer solution
5. After closing the glass cover, gently press it with the tip of a pencil, and then observe

Amoeba

1. Prepare glass objects
2. Drip ± 1-2 drops of material onto the glass of the object by means of a pipette and then carefully cover with a glass cover
3. Observe under a microscope that begins with the use of a weak magnification. Amoeba looks like a translucent and relatively shiny "small mass" shape
4. For closer observation after the object is found, add magnification by turning the micrometer or turning the revolver to a larger magnification on the microscope
5. Draw the shape of the amoeba structure and name the parts, and color them according to the color on the amoeba that you obtained in the observation
6. Draw the amoeba movements you observe about the way the movements are carried out by preparation more than one image and marking the arrows of the observed direction of movement of the amoeba.

OBSERVATIONS

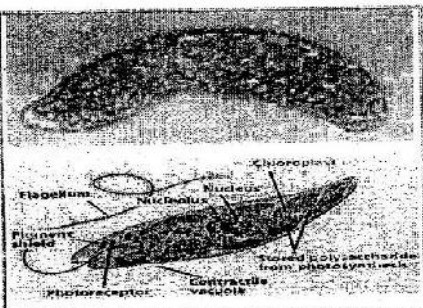
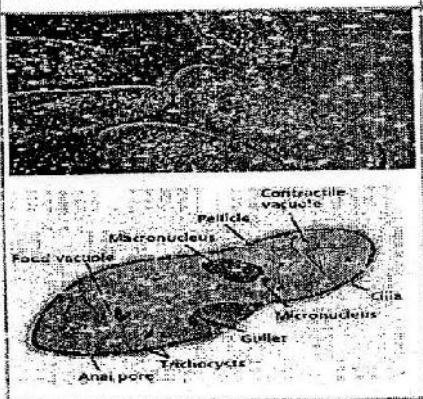
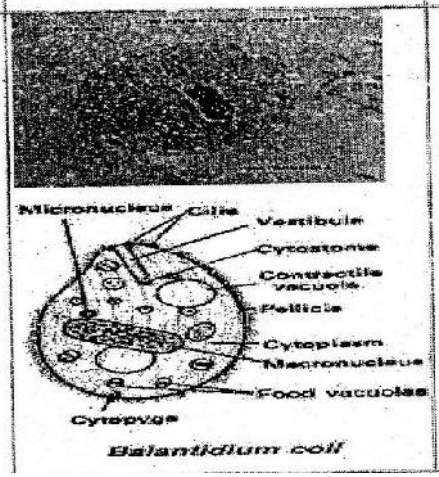
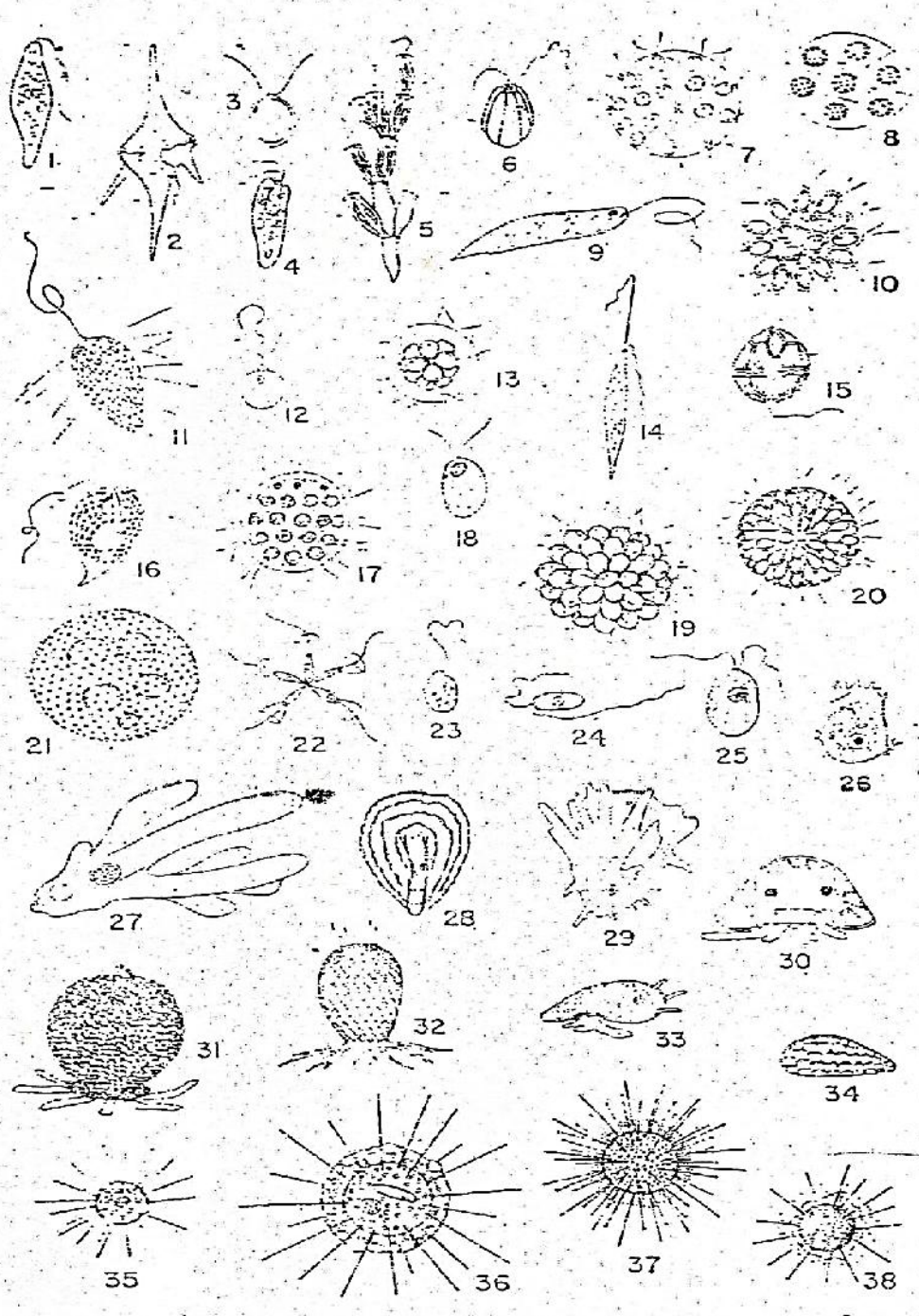
Euglena sp		Divisio : Euglenophycota Kelas : Euglenophyceae Ordo : Euglenales Familia : Euglena Genus : Euglena Spesies : *Euglena sp.*
Paramecium sp		
Balantidium coli		

TABLE II A: PROTOZOANS

Figs.	*Genera*	*Length in μ*	*Remarks*
37.	Acanthocystis	40–100	Usually greenish color, several species
35.	Actinophrys	40–50	Among aquatic plants, often greenish, called "Sun animalcules"
36.	Actinosphaerium	200–300	Among aquatic vegetation
27.	Amoeba	to 600	Fresh or salt water; damp soil
30.	Arcella	30–100	Many species; stagnant water, in bottom ooze and damp soils
1.	Astasia	50–60	Stagnant water; colorless
24.	Bodo	10–15	Stagnant water, often encysted
33.	Centropyxis	100–150	With finger-like cytoplasmic projections; sand grains on body
2.	Ceratium	100–700	Numerous species; fresh and salt water; great color variation
4.	Chilomonas	20–40	Colorless; stagnant water; in bottom ooze and decaying vegetation
3.	Chlamydomonas	10–30	Common in lakes; green
22.	Codosiga	15	Attached by stalks to plants, debris, etc.
31.	Difflugia	200–230	Sand grains encase body; widespread
5.	Dinobryon	30–44	Often colonial, attached to bottom, chromatophores a yellowish brown color
6.	Entosiphon	20	Body oval, flattened, colorless
7.	Eudorina	40–150	Colonies of 16–64 cells; in ponds, lakes and ditches; green
8.	Eudorina	40–150	Colony undergoing asexual reproduction
9.	Euglena	33–55	Abundant in stagnant water with algae. May form green scum on water
32.	Euglypha	20–160	Body covered with siliceous scales
10.	Gonium	90	Forms disc-like colonies, 4–16 cells arranged in single plane; green
26.	Hartmanella	20	A typical soil amoeba
11.	Mallomonas	50	Body covered by siliceous scales
29.	Mayorella	50–300	An amoeba with characteristic tapering pseudopodia
12.	Monas	14–16	Motile in decaying vegetation; colorless
25.	Naegleria	20	Flagellate stage in life cycle of soil-dwelling amoeboflagellate
23.	Oikomonas	5–20	Stagnant pools and soils; colorless
13.	Pandorina	20–50	In spherical colonies of 8–32 cells in gelatinous mass; ponds and ditches; green
14.	Peranema	40–70	Stagnant water; colorless
15.	Peridinium	44–48	Numerous species; brownish chromatophores
16.	Phacus	40–170	Body flattened and often ridged; many species; very common; green
17.	Pleodorina	450	32, 64 or 128 cells per colony. Somatic and reproductive cells separate; green
18.	Polytoma	15–30	Decaying vegetation in stagnant water; colorless
19.	Synura	100–400	Spherical colonies. Said to impart odor of cucumbers; lakes; golden brown
28.	Thecamoeba	200	An amoeba with a rigid pellicular covering
34.	Trinema	30–100	Among aquatic vegetation
20.	Uroglena	40–400	In ovoid or spherical colonies; gelatinous processes join individual cells
38.	Vampyrella	30–40	Often a bright orange color; predatory on filamentous algae
21.	Volvox	350–500	Colonies of many cells form a hollow ball; green

PLATE IIA: PROTOZOANS



1. Astasia
2. Ceratium
3. Chlamydomonas
4. Chilomonas
5. Dinobryon
6. Entosiphon
7. Eudorina
8. Eudorina
9. Euglena
10. Gonium
11. Mallomonas
12. Monas
13. Pandorina
14. Paranema
15. Peridinium
16. Phacus
17. Pleodorina
18. Polytoma
19. Synura
20. Urogiena
21. Volvox
22. Codosiga
23. Oikomonas
24. Bodo
25. Naegleria
26. Harrisonella
27. Amoeba
28. Thecamoeba
29. Mayorella
30. Arcella
31. Difflugia
32. Euglypha
33. Centropyxis
34. Trinema
35. Actinophrys
36. Actinosphaerium
37. Acanthocystis
38. Vampyrella

TABLE IIB: PROTOZOANS

Figs.	*Genera*	*Length in μ*	*Remarks*
13.	Blepharisma	80–200	Often pinkish in color; in decaying vegetation
29.	Carchesium	100–125	Many species; forms stalked colonies in which individuals contract separately; some are attached to plants and animals
7.	Chilodonella	50–150	Many species; common surface scum of stagnant pools
22.	Codonella	60–70	Body pot-shaped, sharply divided into collar and bowl; collar without spiral structure.
4.	Coleps	50–110	Many species; characteristic plates covering the body.
9.	Colpoda	40–110	In stagnant pools among decaying vegetation
23.	Cothurnia	70–100	Often in gills of crayfish. Attaches to substrate by short stalk
2.	Dictyostelium	variable	A cellular slime mold; cells of plasmodium distinct, developing into a single sporangium
8.	Didinium	80–200	Predaceous on *Paramecium*
6.	Dileptus	250–500	Many species; with neck-like extension of body
24.	Epistylis	50–250	Colony of many individuals united in a multistalk, not contractile; same species occurs on crayfishes and turtles
18.	Euplotes	90	With isolated groups of compound cilia (cirri)
11.	Frontonia	150–600	Among filamentous algae
20.	Halteria	25–50	Performs bouncing movements. Common in pond-water infusions
31.	Ichthyophthirius	100–1,000	Causes "white-spot" or "Ich" disease of fish in aquaria and fish hatcheries
5.	Lacrymaria	500–1,200	Anterior end extensible and highly flexible
28.	Loxodes	700	Strongly compressed; brownish
16.	Metopus	90–140	In decaying vegetation
17.	Oxytricha	50–250	With marginal rows of cirri
10.	Paramecium	100–350	Many species; very common
1.	Physarum	variable	A true slime mold; amoeba-like cells fuse to form multinucleate plasmodium followed by stalked sporangia
26.	Podophrya	10–100	With sucking tentacles, stalked; close to *Tokophrya*
3.	Prorodon	30–130	A typical, primitive ciliate
14.	Spirostomum	1,000–3,000	One of largest protozoans, highly contractile
15.	Stentor	1,000–2,000	Attached or free-swimming; trumpet-like shape
30.	Stylonychia	100–300	Many species
27.	Tokophrya	50–175	With free-swimming ciliated young; adult stalked, non-ciliated, and bears sucking tentacles
12.	Urocentrum	50–80	Among pond vegetation
19.	Urostyla	200–600	Many species; numerous rows of cirri
22.	Vorticella	135–150	Attaches to substrate by contractile stalk; with free-swimming stage
25.	Zoothamnium	250	Colony of many individuals united in a common stalk; contracts as unit; colonies several mm. high

REFERENCES: PROTOZOANS

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1953 *Protozoology*. Prentice-Hall, Inc., New York, N.Y., 682 pp.

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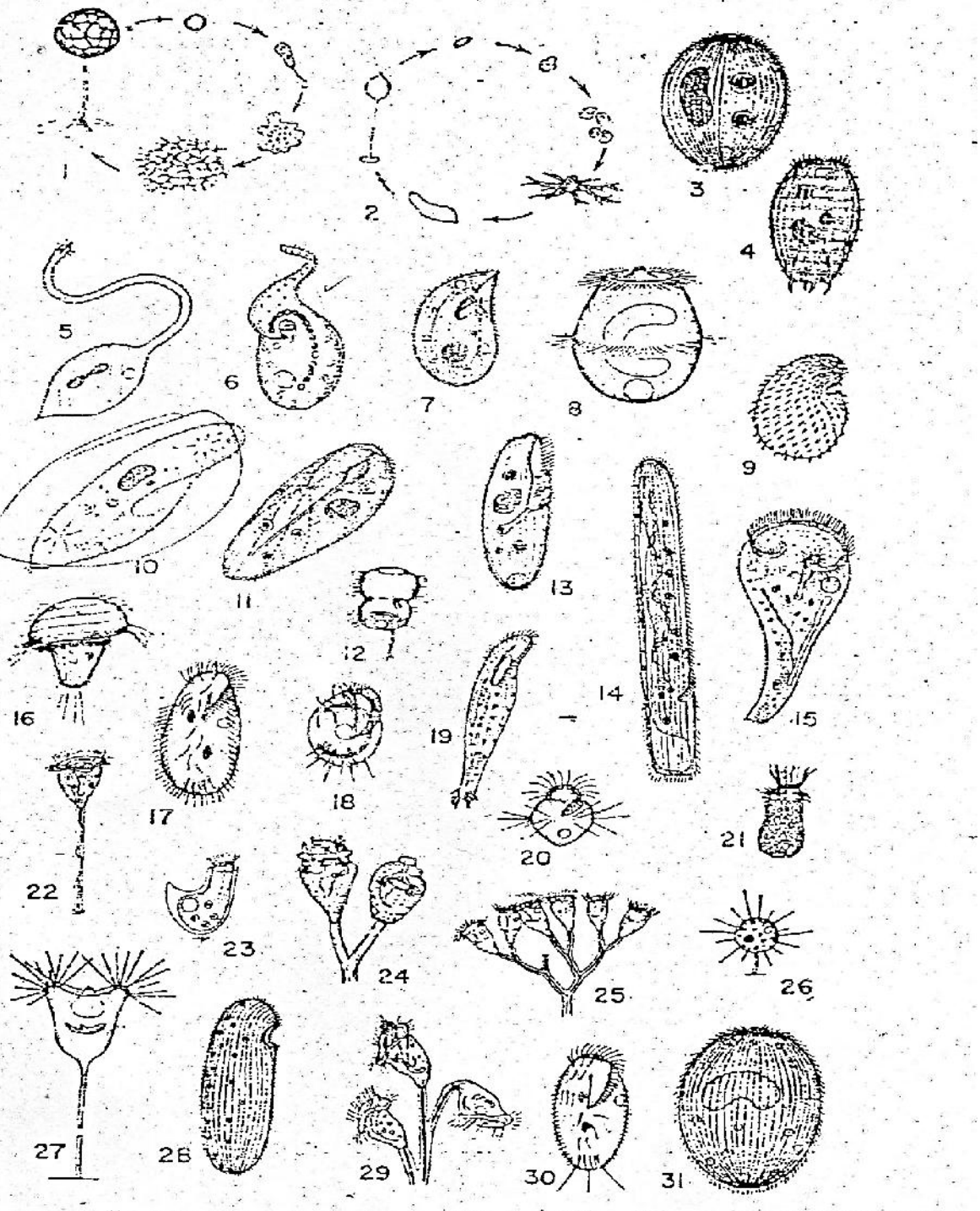
1949 *How to know the protozoa*. Wm. C. Brown Co., Dubuque, Iowa, 234 pp.

Kudo, Richard R.
1954 *Protozoology*. 4th Ed. C. C Thomas, Springfield, Ill., 966 pp.

PLATE IIB: PROTOZOANS



1. Physarum
2. Dictyostelium
3. Prorodon
4. Coleps
5. Lacrymaria
6. Dileptus
7. Chilodonella
8. Didinium
9. Colpoda
10. Paramecium
11. Frontonia
12. Urocentrum
13. Blepharisma
14. Spirostomum
15. Stentor
16. Metopus
17. Oxytricha
18. Euplotes
19. Urostyla
20. Halteria
21. Codonella
22. Vorticella
23. Cothurnia
24. Epistylis
25. Zoothamnium
26. Podophrya
27. Tokophrya
28. Loxodes
29. Carchesium
30. Stylonychia
31. Ichthyophthirius

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	THE UNIVERSITY OF JEMBER FACULTY OF MATHEMATICS AND NATURAL SCIENCES DEPARTMENT OF BIOLOGY STUDY PROGRAM OF BACHELOR BIOLOGY	CODE F1.03.06

STUDENT ASSIGNMENT PLAN

COURSE	Microbiology				
CODE	MAB 1201	Credits	3 (2-1)	SEMESTER	2
LECTURERS	Dr. Sutoyo, M.Si, Drs. Rudju Winarsa, M.Kes				

TASK FORM

Case method

TASK TITLE

Study of the structure and diversity of acellular microorganisms as the basis for the treatment of diseases caused by viruses

SUB-ACHIEVEMENTS OF LEARNING COURSES

Students able to describe the morphological diversity of microorganisms (3a)

TASK DESCRIPTION

Indonesia is experiencing the Covid-19 pandemic that has paralyzed all areas of life. Covid-19 is a disease caused by the coronavirus. In addition to Covid-19, some diseases caused by viruses such as hepatitis (*Hepatovirus A*), measles (*Pramixovirus*), rabies (*Rabies lyssavirus*), polio (*Enterovirus C*), herpes (*Herpervirus*), chickenpox (*Varicella zoster virus*) and mumps (*Mumps virus*). The government has tried to provide treatment in the health sector to overcome the spread of the disease. Your role as a prospective Biology graduate who is taking a Microbiology course is needed to help the development program for handling some of these diseases. Students as prospective microbiologists are expected to play a role by providing scientific information by presenting a review of basic knowledge about the virus that causes disease and its explanation of the biology of the virus as a potential to produce ideas for its control to the community in the future. The information is arranged in the form of posters which include: 1) what are the characteristics of microscopic structures, life cycles, mechanisms of infection, and symptoms of diseases by viruses and 2) the information is also enriched with the idea of how to handle the virus that causes the disease with an explanation of which characteristics have the potential to be inundated the effective impact of the pandemic.

METHODS OF WORKING ON TASKS

Poster arrangement

OUTER SHAPE AND FORMAT

Object of work:

Understanding knowledge of the biology of disease-causing viruses to explore ideas aboutthe management of the disease

Outcome shape:

Posters are arranged with the appropriate format and content in the rubric

ASSESSMENT INDICATORS, CRITERIA AND WEIGHTS

1. Indicators and assessment criteria according to the rubric.
2. The assessment weight for poster assignments is 20% of the total lecture value

SCHEDULE

1. March 28, 2022: Group division
2. March 28 – April 11, 2022: In-class *breakout room discussions* and self-paced discussions outside the class schedule
3. April 12, 2022: Individual assignment collection view assignment schedule in SISTER

MISCELLANEOUS

1. The activeness in the discussion of the preparation of posters for each group was recorded and uploaded in Kawanda and the Kawanda link was written in this column: ……..
2. RTM Poster Virus is uploaded individually in MMP SISTER with the format according to the rubric in the form of a PDF file combined with the RTM page and this rubric.

REFERRAL LIST

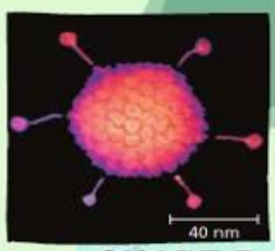
Madigan, M.T, J.M Martinko and J. Parker. 2019. Biology of Microorganisms. Prentice-Hall

Group 04
Microbiology B

HUMAN ADENOVIRUS

WHAT IS HADV?

- Is a DNA virus that is icosahedral, unenveloped, and has a size of 90-100 nm.
- Is a virus that causes respiratory disorders, stomach, intestinal, and eye infections in humans.
- Penetrate into host cells.
- Has 3 main protein components and has a 36-38 kb DNA genome.

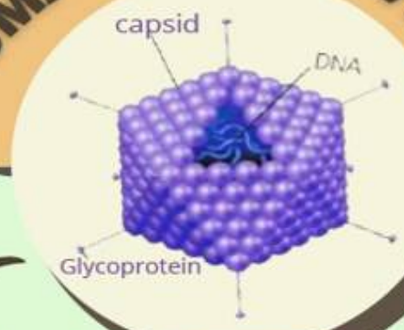


HAdV under the electron microscope

SHAPE & STRUCTURE

- Polyhedral body shape
- Has cube symmetry
- There are 12 capsomeres which are either icosahedrons (angular shape with 20 sides) or a combination

SYMPTOM



HAdV

LIFE CYCLE

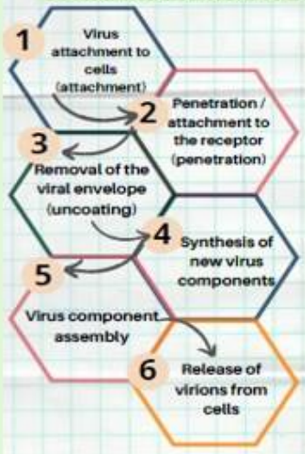
Lytic Cycle

1. Virus adsorption is characterized by the attachment of the virus to the cell wall
2. In bacteriophage viruses, penetration is done by perforating the cell membrane using enzymes.
3. Viruses make use of cell organelles.
4. Cells undergo lysis.

Lysogenic Cycle

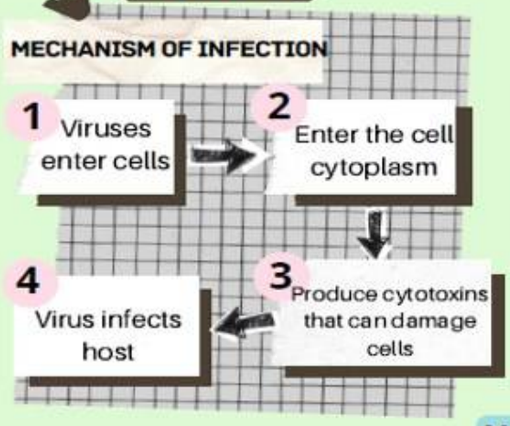
The genetic material of the virus and the host cell combines.

Virus Replication Stage



PREVENTION

MECHANISM OF INFECTION



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Supraptо, S. 2017. Hubungan sanitasi lingkungan dengan kejadian diare pada lanjut usia di Kelurahan Barombong Kecamatan Tamalate Kota Makassar. *Jurnal Ilmiah Kesehatan Sandi Husada* 6 (2):51-68.

MEMBER'S NAME

1. Mahira Atla Amadea (211810401049)
2. Yuli Isti Mulyani (211810401050)
3. Dwiki Haykhal Satriawan (211810401047)
4. Siti Afidatu Rohmah (211810401051)
5. Qoidah Anis Mahiroh (211810401052)

RUBRIC FOR CASE METHOD

Course/Code	: Microbiology
Group Number	: 04
Student Name	: Mahira Atla Amadea (211810401049) Yuli Isti Mulyani (211810401050) Dwiki Haykhal Satriawan (211810401047) Siti Afidatu Rohmah (211810401051) Qoidah Anis Mahiroh (211810401052)

Course learning outcome: students able to use bioscience in solving problems related to biological resources in tropical environments students conduct case analysis to build solution recommendations, assisted by group discussions to test and develop design solutions;
Each Course can use criteria 1-5 according to the type of problem-based learning/case method implemented

No	Criteria	1 (<50)	2 (50-60)	3 (61-70)	4 (71-80)	5 (80<)	Weight	Score
1.	Identification of the main issues and/or problems indicated by: a) New topic issue/recently happening b) Problem identification c) Understanding the problem	Does not fulfill requirement indicators	Fulfill one requirement indicators	Fulfill two requirement indicators	Fulfill three requirement indicators	Excellent, Fulfill four requirement indicators		

	d) Complete problem statement using Indonesian Standard							
2.	Literature research & review	**Use <2 Good** national journal article within <5 years publication	**Use <2 Good** international journal article within <5 years publication	**Use 2-3 Good** international journal article within <5 years publication	**Use >5 Good** international journal article within <5 years publication	**Use >5 Excellent** international reputation journal article within <5 years publication		
3.	Analysis of the key issues	the analysis does not meet all the criteria required by the course	Incomplete analysis	Insightful and thorough analysis of **some** the key issues required by the course	Insightful and thorough analysis of **most** the key issues required by the course	Insightful and thorough analysis of **all** the key issues required by the course		
4.	Alternative solutions and/or options		analysis of alternative solutions does not cover the main issues proposed	Less comprehensive analysis alternative solutions cover some the main issues proposed	Comprehensive analysis of alternative solutions covers most the main issues proposed	Comprehensive analysis of alternative solutions cover all the main issues proposed		
5.	Case Report (Video, Resume,	Does not fulfill any	Fulfill one point format	Fulfill some points format	Fulfill most points format	Excellent, fulfill all points	100%	80

	Article, presentation, poster)	point format structure required by the course	structure required by the course	structure required by the course	structure required by the course	format structure required by the course		

	THE UNIVERSITY OF JEMBER **FACULTY OF MATHEMATICS AND NATURAL SCIENCES** **DEPARTMENT OF BIOLOGY** **STUDY PROGRAM OF BACHELOR IN BIOLOGY**	**CODE** **FORM PP-05**

STUDENT WORKSHEETS

Course Lecturer : Dr. Esti Utarti, S.P., M.Si. & Drs. Siswanto, M.Si.
Subject Matter : Isolation of Microorganisms
Learning Model : Practical in the Laboratory (Activity Observation and Assessment of Practical Reports)

STUDENT IDENTITY	
Name/ID/Class	Maulidya Dwi Nuraini /211810401070/C
Group Member Name	1. Refi Khoirotul Aini/ 211810401068 2. Rahma Hanifa/ 211810401069 3. Nanda Rexgina Putri/ 212810401071 4. Sealfiana Permata Sari/ 211810401075 5. Selly Hervianingsih Ramadhani/ 211810401077 6. Mei Cahyani Salsabila/ 211810401080 7. Suhartatin/ 211810401082
Meeting on	5 (fifth)
Day/Date	Friday, April 1, 2022

Practicum Activities

1. Learn microorganism isolation techniques according to practicum instructions and assistance by Lecturers / Assistants
2. Practicum instruction resources are available in SISTER
3. Carry out microorganism isolation activities which include:
    a. Checking the availability of tools and materials
    b. Perform practicum isolation techniques
    c. Record and observe practicum results
4. Create a practicum report

RESULTS OF PRACTICUM ACTIVITIES

MICROBIAL ISOLATION

MICROBIOLOGY PRACTICAL REPORT

COMPILED BY
NAME : MAULIDYA DWI NURAINI
SIN: 211810401070

STUDY PROGRAM OF BACHELOR IN BIOLOGY
DEPARTMENT OF BIOLOGY
FACULTY OF MATHEMATICS AND NATURAL SCIENCES
UNIVERSITY OF JEMBER
2022

1. INTRODUCTION

Microorganisms in a natural environment are mixed populations of various types, both microorganisms present in the air, soil, water, and food. Microbial separation aims to find out the type, culture, morphology, physiology, and characteristics. The separation technique is called isolation which is accompanied by purification. Isolation is a process or way to separate certain microbes from their environment by growing them in an artificial medium so that an unmixed culture is obtained (pure culture) of other types of microbes. The principle of microbial isolation is to separate one type of microbe from another that comes from a mixture of various microbes. This treatment is carried out on a solid medium, so that microbial cells will form colonies of cells that remain in place (Sabbathini et al., 2017). Microbes presenting in nature are a mixture of various species. The method of obtaining a pure culture of such a mixed culture, is used by the method of pouring plates and the method of streak plates. According to Angelia (2020) states that the pouring plates method is a microbial isolation technique used to obtain pure colonies of microorganisms. The streak plates method is streaking the suspension of microbial-containing materials on the surface of the medium with L rods (Drafting Team, 2022). Microbial isolation in this practicum uses samples in the form of air, apple surface, and paddy fields. Microbial isolation is prone to failure so the materials needed that support the applied microbial isolation techniques. The solution medium used to maintain the survival of bacterial isolates is a physiological salt solution of 0.85% (m/v). This practicum in each sample is repeated so that the results of microbial isolation can be compared with each other. The purpose of this practicum is that students can isolate and calculate the number of microbes from nature (Rahardhianto et al., 2012).

2. METHOD

2.1 The tools used in microbial isolation practicum are as follows:

- Petri dish
- Seal
- Dorslag paper
- Incubator
- Bunsen lamp
- Match
- Erlenmeyer flask

- Cutton bud
- Test tubes
- Three-arm scales/ balance sheets
- Glass trunk L
- Spoon
- Label
- Vortex mixer
- Micropipette
- Microtip

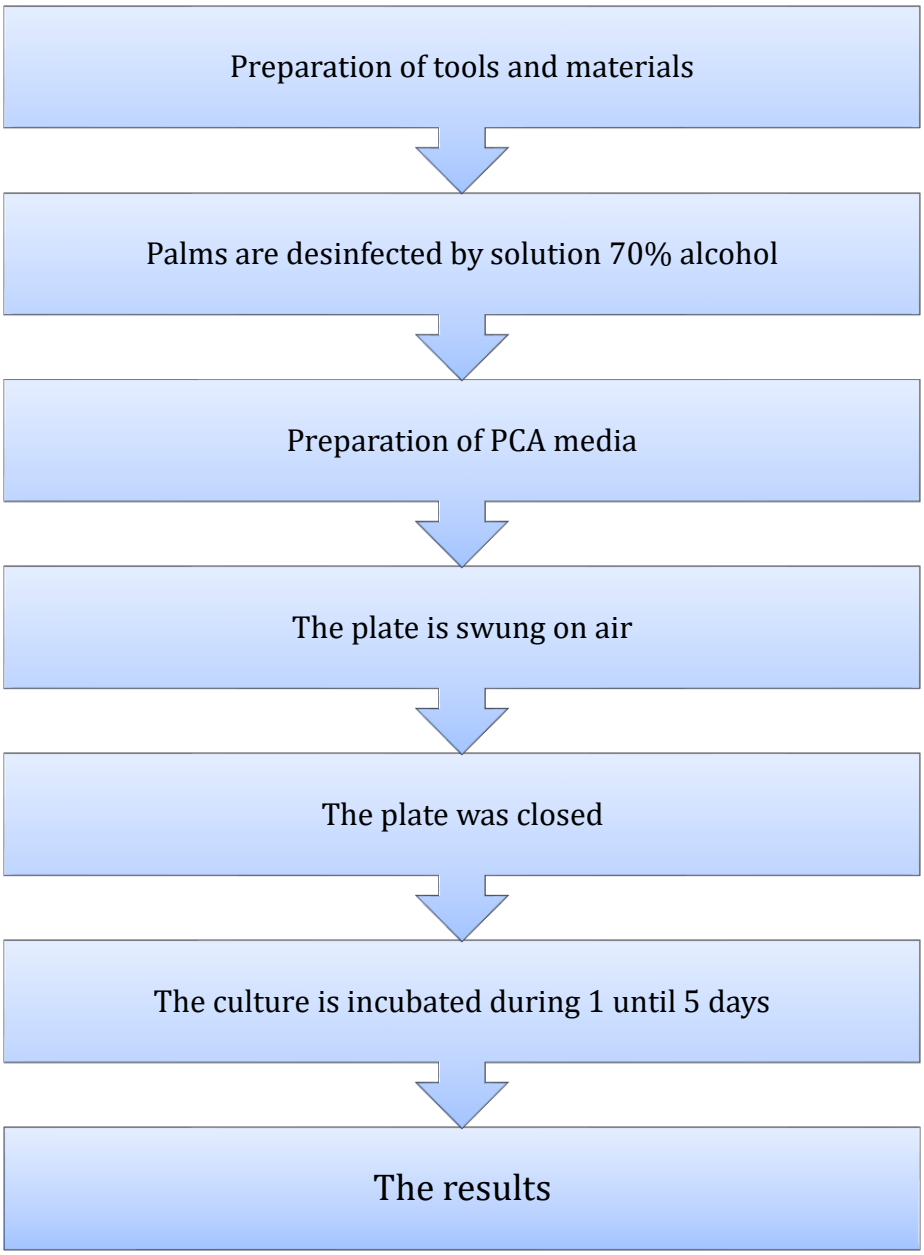
2.2 Materials used in microbial isolation are as follows:

- Alcohol 70%
- Air
- Spirtus
- Red apple
- Soil samples 10%
- Physiological saline solution (NaCl) 0,85%
- Solid and liquid PCA (Plate Count Agar) media

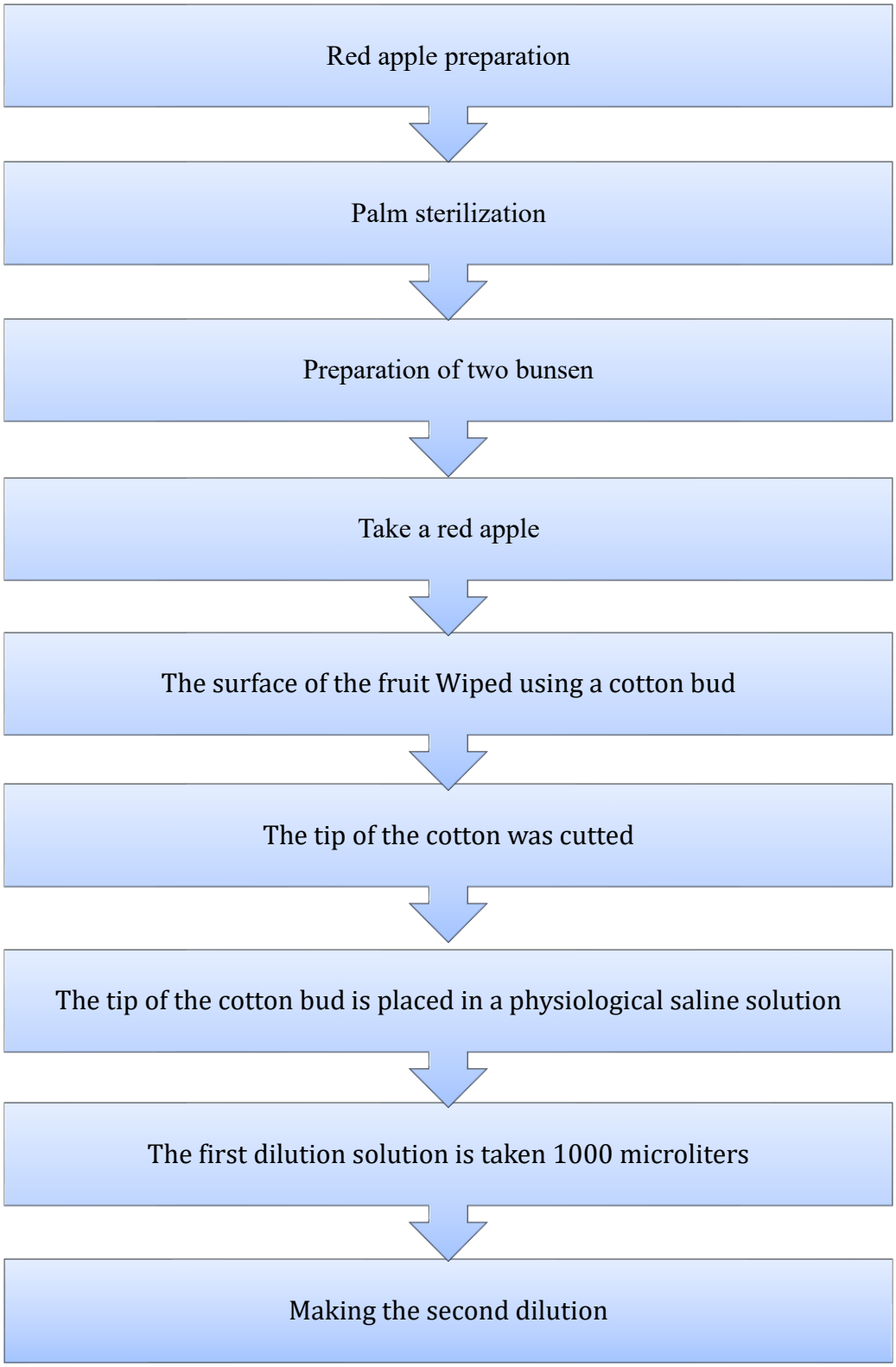
2.3 Procedure

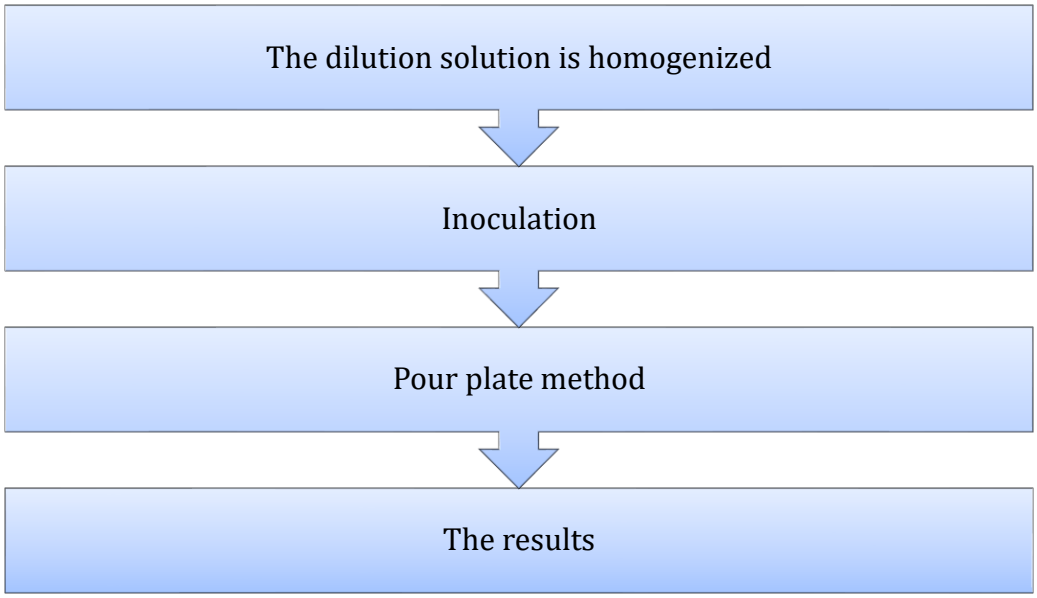
The work steps used in the microbial isolation are as follows:

2.3.1 Air Samples

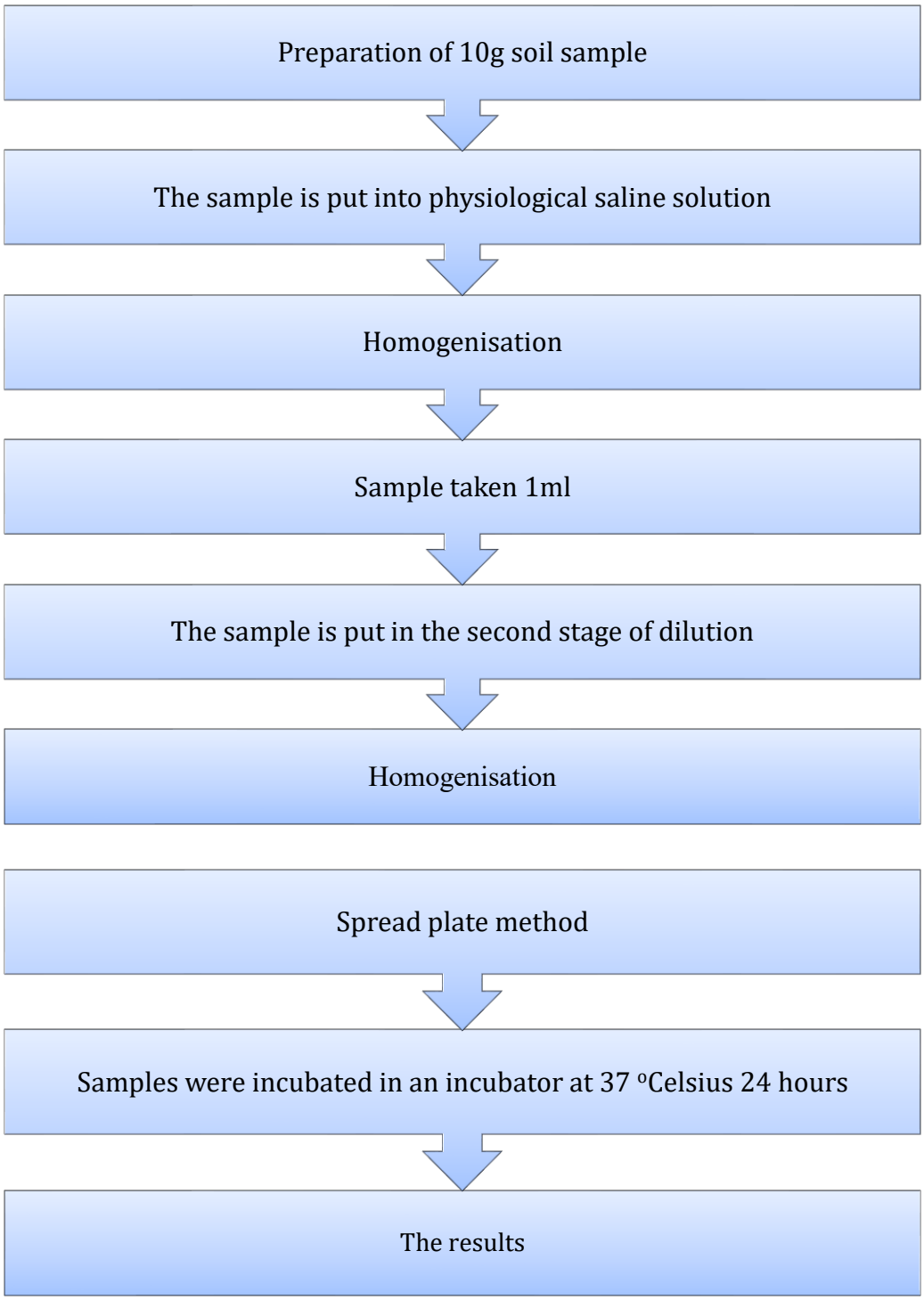


2.3.2 Sample Wipe of Red Apple Skin (Pour Plate)




The dilution solution is homogenized
Inoculation
Pour plate method
The results

2.3.3 Soil Sample



3. RESULTS AND DISCUSSIONS

3.1 Results

3.1.1 Table 1: Microbe was isolated from air sample

Location	**Replication**	**Isolates**			
		Bacteria	**Actinomycetes**	**Yeast**	**Fungi**
Outdoor	I	2	-	-	10
	II	-	-	-	11

3.1.2 Table 2: Microbe was isolated from the red apple sample

Isolates	Dilutions		
	10^{-2}	**10^{-3}**	**10^{-4}**
Bacteria	16	uncountable	3
Actinomycetes	-	-	-
Yeast	-	-	-

3.1.3 Tabel 3: Microbe was isolated from soil sample

Isolates	**Dilutions**		
	10^{-2}	**10^{-3}**	**10^{-4}**
Bacteria	uncountable	uncountable	2
Actinomycetes	-	-	-
Yeast	-	-	-
Fungi	uncountable	3	1

*Uncountable (number of microbe isolates >100).

3.2 Discussion

The practicum carried out this time is microbial isolation. The purpose of this practicum is to know how to isolate and calculate the number of microbes from nature. This practicum is carried out with several samples, namely air, fruit surface (red apple), and soil. This practicum is supported by various materials to maintain the survival of bacterial isolates, which is a 0.85% physiological salt solution, and is bred on PCA (Plate Count Agar) media. This microbial isolation practicum also pays attention to the sterility of using the tool and also the workspace, which is to use 70% alcohol, so that other microbes will not stick to the media to be used.

Microbial isolation is a way to separate one type of microbe from another from various microbial mixtures with the aim of obtaining a pure culture. Pure culture is obtained by performing a stratified dilution of the sample. According to Putri and Kusdiyanti (2018) states that identifying microbes aims to find out the morphological properties, biochemical reactions, and analyze of the molecular number of bacteria from nature. Microorganisms is very diverse in its types, some are in the soil, water, air, food, and those found in the body of animals and plants. Microorganisms have an important role in the life cycle, especially as decomposers also play a negative role in becoming pathogens. According to Nugraha et al (2017) the principle of microbial isolation is the separation or transfer of certain microbes, which are grown through artificial media so that pure culture is obtained. Pure culture is a culture in which microbial cells are derived from the division of a single cell. Pure culture is useful for studying and identifying microorganisms and growing them as pure culture material in artificial media. The culture grown in the solid medium causes the cells of the microbes to form a colony of cells that remain in place. The calculation of bacteria in the media dish has the principle that the microscopies body cells that are still alive in the media in order, then the cells multiply and can be counted or seen directly without using a microscope (Nurtjahyani, 2014).

Microbial isolation techniques or methods are divided into three, namely the streak plate method, the pour plate method, and the spread plate or spread method. The streak plate method is a method by using an ose loop that is scratched to the surface of the media in order to be with a certain pattern. The pattern leaves a single bacterial cell attached to the medium. Such single bacterial cells can form colonies that can be moved to obtain a pure culture. This scratch method, if done well, can cause the isolation of the desired microorganisms. The pour plate method is a method of mixing a bacterial suspension with a agar medium at a temperature of 50oC and pouring it on a petri or Petri dish. The hardened agar medium causes the bacteria to stick and cluster so that a single colony is formed. The spread or spread plate method is a method that is carried out by pouring a suspension of bacteria onto the medium so that it then spreads it evenly using L glass. This method aims to separate bacteria individually and can grow into a single colony (Nugraha et al., 2017).

The first sample carried out in this practicum was using air samples outside the microbiology laboratory. The tools and materials needed in the air sample are Petri dish with compacted PCA (Plate Count Agar) media, Bunsen, seal and dorslag paper, as well as outdoor air. The first treatment was to open the dorslag paper as a Petri dish wrapper and open the Petri dish seal. The Petri dish to be used is sterilized on the Bunsen fire. This treatment is carried out by heating the edges of the Petri dish and rotating slowly so that the entire Petri dish area is sterile. The purpose of this treatment is so that unwanted microbes on the edges of the Petri dish do not enter the sample. The Petri dish lid is opened and captured outdoor air 5-10 times, that is, swinging in a one-way motion, with the aim that microbes can be caught and attached to the PCA medium. This treatment is carried out with two repetitions so that between treatments can be compared. The captured air microbes are closed again and reheated on the Bunsen fire so that unwanted microbes die. Petri dish covered with seal and wrapped in dorslag paper.

Petri dish cover of air samples is carried out in a sterilized workspace with 70% alcohol. According to Aminin et al (2013) stated that the placement of Petri dish before wrapping needs to be considered, that is, Petri dish must be turned over, this serves to avoid water droplets that may be able to adhere to the walls of the Petri dish cap. The wrapped Petri dish is labeled as a distinguishing sign. Such air samples were incubated for 3 days and observed. The results obtained in the air sample, namely on the first repetition on the Petri dish surface, found 2 bacteria and 10 fungi. Meanwhile, on the second repetition, 11 mushrooms were found. The group of bacteria and fungi is often found in the air. According to Lanjarini et al (2018) stated that humidity and temperature in the air become a growth medium for microorganism types in the form of bacteria and fungi. Fungal colonies in the outdoor air microbiology are more numerous than bacteria because the morphology of fungal growth is appropriate and is at a temperature and the humidity of the substrate in the high air can suppress the growth of the fungus. High humidity is one of the main factors, besides that when practicing air samples, the weather conditions are after rain falls so that the humidity is high when capturing microbes.

The second sample carried out in this practicum was to use a sample on the surface of the skin of a red apple. The tools used are Petri dish, Bunsen, liquid PCA (Plate Count Agar) media, Erlenmeyer flask, test tubes, micropipettes and microtips, seal and dorslag paper, and a 0.85% graphic solution. The first step is to sterilize the workbench and hands with 70% alcohol and wipe the table using a tissue. Both Bunsens lamp are lit in a vertical direction. Fruits of red apples and sterile cutton bud are prepared. Cutton bud is used to wipe half the surface of the red apple skin so that microbes stick to the tip of the cutton bud cotton. The swapped cutton bud is cut off only the cotton part and put into a test tube with a label of 10-1 which contains a 0.85% physiological salt solution of 9 ml. This treatment aims to ensure that the microbes in the cutton bud can grow and be maintained because they are in accordance with the pH in the graphics solution of 0.85%. According to Sadimin (2016) graphic solution or graphic sodium is an

isotonic solution that has many uses. A 0.85% physiological saline solution is used to maintain the survival of bacterial isolates.

Muniformizes the reagents used as a test of sample activity. The next stage is to move the first dilution sample (10^{-1}) into a test tube 10^{-2}. The solution is taken as much as 1 ml with micropipettes and microtips. According to Rohmatningsih (2021) micropipette is a tool to move a solution or liquid from one place to another with a very small volume and has accurate accuracy and precision. The micropipette can be adjusted in volume as desired to the limit of the pipette's volume scale. The next treatment in the vortex is again to be homogeneous. This treatment is carried out until the fourth dilution or 10^{-4} . A solution of 10^{-2} to 10^{-4} of each dilution for inoculation. A solution of 1 ml of each dilution into sterile Petri dish and prepared PCA media in a liquid state. Previously, the PCA medium was in a lukewarm state of freezing so it had to be heated until it thawed. The function of adding PCA media is as a microbial growing medium. Petri dish was closed again and moved 20 times by forming the number 8. Petri dish is set on a Bunsen fire. Petri dish is covered with seal and dorslag paper and labeled. Wrapped Petri dish incubated in incubator for 3 days.

Result

The results obtained on the Petri dish surface of red apple samples were in the repetition of $10^{-2,}$ 16 bacteria and 8 fungi. Repetition of 10^{-3} , bacteria cannot be counted because their number >100. Repetition of 10^{-4} , produces as many as 3 bacteria and 2 fungi. The more degree of dilution or repetition present on the Petri dish surface will result in fewer and fewer microbes. This is due to the multistage dilution which aims to reduce or reduce the number of microbes contained in the liquid (Widyorini et al., 2016).

The third sample carried out in this practicum is to use a sample of paddy fields. The tools and materials used are Bunsen, Petri dish and PCA media, Erlenmeyer flask, test tube, L rod, spirtus, three-arm balance, vortex, and soil. The first stage is to sterilize the workbench and hands practice with 70% alcohol. Both Bunsen fires are ignited and placed vertically. Soil samples were weighed with a three-arm balance of 10 grams. A soil sample is introduced on an Erlenmeyer flask containing a 0.85% graphical solution with a volume of 90 ml, and is considered the first dilution or repetition (10^{-1}). Erlenmyer flasks are vortexed to be homogeneous. The solution is taken in the amount of 1 ml with micropipettes and microtips, then it vortexed returns and is characterized by dilution or a second repetition (10^{-2}). This treatment is repeated until the fourth dilution or repetition (10^{-4}). The results of the second to fourth dilutions are put into a Petri dish containing PCA media to culture the microbes. The result of the dilution is 1 ml each with micropipette. The next step is to take the L stem and sterilize it by dipping the tip into the spiritus then heat it on the Bunsen fire. Cool or wait for the L rod so that it can be used. Petri dish was scratched until it was evenly distributed with the L rod and after it was deemed sufficient, it was

rotated 90 degrees and scratched again on the surface of the media. It is re-closed and the edges of the petri dish are re-lit, covered with seal, and wrapped in dorslag paper. Petri dish containing soil samples incubated with an incubation for 3 days and the results were observed.

The results of observations on soil samples, namely in the second repetition (10-2) colonies of bacteria and fungi cannot be counted because the number of colonies > 100. On the second repetition (10 -3) the colonies of bacteria could not be counted and the fungus as many as 3. On the second repetition (10-4 colonies of bacteria as many as 2 and fungi only 1. Microbes in the soil in large quantities have a role as soil fertilizers because they produce nutrient substances in the soil. In addition, microbes also play a role in decomposing organic materials and producing substances that can inhibit microbial growth as a result of their metabolism (Abdulbasith et al., 2020).

4. CONCLUSION

This practicum can be concluded that microbial isolation is a way to separate one type of microbe from another from various kinds of microbial mixtures with the aim of obtaining pure culture. Microbial separation aims to find out the type, culture, morphology, physiology and characteristics. The culture grown in the solid medium causes the cells of the microbes to form a colony of cells that remain in place. Microbial isolation techniques or methods are divided into three, namely the streak plate method, the pour plate method, and the scatter or spread method The sample in this practicum is a pure culture obtained by doing a stratified dilution of the sample. So that the more the degree of dilution or repetition that is on the surface of the Petri dish will result in fewer microbes. Graded dilution aimed at reducing or reducing the number of microbes present in the liquid. The success of carrying out microbial isolation of a material is not only supported by the applied insulation techniques. Various other factors that support the success of isolation include the type of medium, incubation conditions, and the addition of antibiotic compounds.

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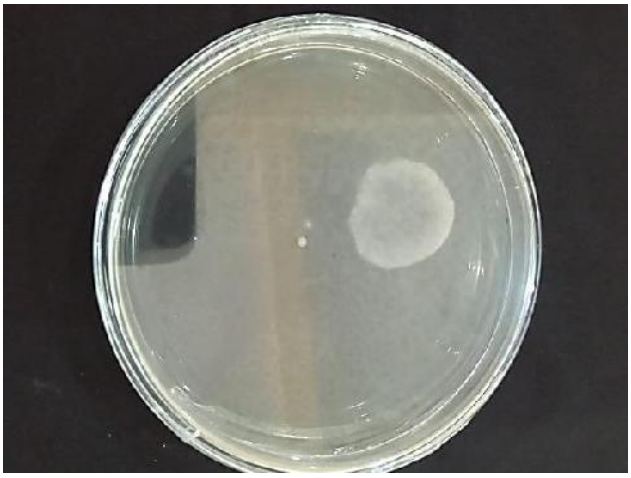
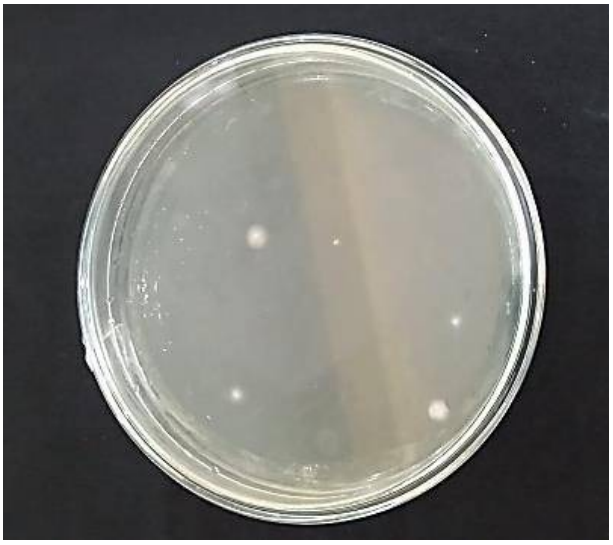
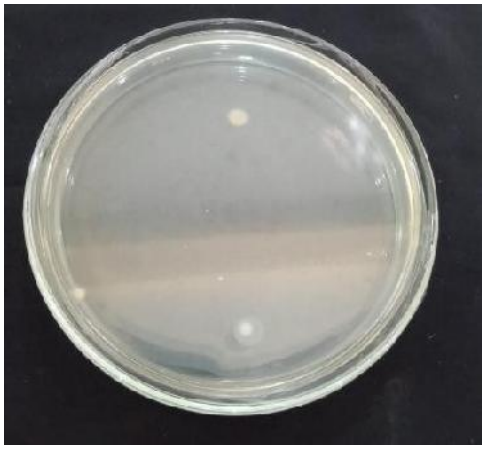
ATTACHMENT

1. Table of microorganisms isolated from the air

Sample	Replication	Picture
Air from outside the laboratory	1	
	2	

2. Table of microorganisms isolated from fruit surface

Sample	Dilutions	Picture

	Red apples	10^{-2}	
		10^{-3}	
		10^{-4}	

3. Table of microorganisms isolated from the soil sample

Sample	Dilutions	Picture
Paddy fields	10^{-2}	
	10^{-3}	
	10^{-4}	

RUBRIC FOR LABORTORY-FIELD WORK

Course/Code	: MAB 1201
Group Number	: 2
Student Name	: Maulidya Dwi Nuraini /211810401070/C

No	Criteria	1 (<50)	2 (50-60)	3 (61-70)	4 (71-80)	5 (80<)	Weight	Score	Total
1	Literature, indicated by: a) The references used (textbook, article journal, proceeding) are within <10 years publication b) All references used are stated in the manuscript c) The references are cited using appropriate sentence d) Using primary source for references e) The references used are in accordance to the topics discussed	Fulfill only one criteria required by the course	Fulfill two criteria required by the course	Fulfill three criteria required by the course	Fulfill four criteria required by the course	Fulfill all criteria required by the course	20%	80	16
2	Report (Writing report)	Does not fulfill any	Fulfill one point	Fulfill some	Fulfill most points	Excellent, fulfill all	80%	81	64,8

		point format structure required by the course	format structure required by the course	points format structure required by the course	format structure required by the course	points format structure required by the course			
									80,8

Module designation	*: **Plant Systematic***
Semester(s) in which the module is taught	*: odd/IV*
Person responsible for the module	1. *Dra. Dwi Setyati, MSi.,* 2. *M. Su'udi, PhD.* 3. *Dr.rer.nat. Fuad Bahrul Ulum, S,Si. M.Sc*
Language	: *Bilingual*
Relation to curriculum	: *Compulsory / ~~elective~~ / ~~specialisation~~*
Teaching methods	*: Lecture- Discussion, Project, Presentation, Practical course*
Workload (incl. contact hours, self-study hours)	*(Estimated) Total workload:*181.32*hr* a. *Lecture-Discussion*: 102*hr* b. *Laboratory work and Field work* a. *Laboratory work:28,2hr* b. *Field work: 17hr* c. *Project (herbarium): 17hr* d. *Presentation: 17hr*
Credit points	*: 4 credits or 6.04 ECTS*
Required and recommended prerequisites for joining the module	*: Plant Structure*

Module objectives/intended learning outcomes	*Knowledge: able to analyze the principle of molecular biology, cells, and organism (LO 3)* ● *able to describing the principles of plant systematics concepts (LO 3.a)* *Skills: able to **implement** biological concepts in laboratory work and/or field studies independently and/or in groups (LO 6)* ● *able to practicing laboratory work both independently and in groups to demonstrate the principles of plant systematics concepts (LO 6.a)*
Content	*This course **describes concepts** of Plant systematic: Describe the principal concept of plant taxonomy, herbarium and its curation, evolution and phylogeny.* *There are also implementation scientific methods for Plant systematic through **a Project-based Method** by observing the plant collection of botanical garden then the result will be presented as book report. The second task is submitting a complete and correct specimen of herbarium.*

Examination forms	1. *Essay test* (25%) 2. *Fill in the blank* (10%) 3. *Project based method:* *a.* Progress report (5%) b. Final report (10%) 4. *Laboratory work and Field Work (25%+25%):* *a. Laboratory:* *i.* Post test(4%) ii. Responsi (9%) iii. Activity observation (5%) iv. Report (7%) *b. Field work* i. Attitude (5%) ii. Progress report (5%) iii. Final report (5%) iv. Report presentation (5%)
Study and examination requirements	*: passing grade 70%* *Requirements for successfully passing the module*

Reading list	1. Cronquist, Arthur. 1981. An Integrated System of Classification of Flowering Plants. Columbia University Press. New York. 2. Simpson, M.G., 2019. *Plant systematics*. Academic press. 3. Stuessy, T.F., 2009. Plant taxonomy: the systematic evaluation of comparative data. Columbia University Press. 4. Holttum, R. E. 1967. A Revised Flora of Malaya Volume II. Ferns of Malaya. Government Printing Office. Singapore. 5. Levetin & McMahon. 2008. Plants and Society, Fifth Edition Introduction to Plant Life: Botanical Principles Plant Systematics and Evolution. The Mc Graw Hill Companies 6. de Winter, W. P. and V. B. Amoroso. 2003. Plant Resources of South-East Asia Cryptogams: Fern and Fern Allies. Bogor: Prosea Foundation.

Credits to ECTS conversion formula 1 SKS TM = 50min T+60min TS+60min M (170 minutes) x 16 weeks = 45.33 Hours 1 SKS Practice = 170 min. 1 ECTS = 29.99 hours 1 Credit = 1.51 ECTS

Plant Systematics

Practical book

Editors:
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Biology department
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2022

Table of contents

Learning Outcome

The learning outcome of the practical course in Plant systematic are:

Attitude:

Able to internalise norms and ethics based on Pancasila in working independently or in groups (LO 1)

able to show honest attitude and responsibility as the practice Pancasila(LO1a)

Knowledge:

Able to analyze the principle of molecular biology, cells, and organism (LO3)

able to describe the principles of plant systematics concepts(LO3a)

Skills:

Able to implement biological concepts in laboratory work and/or field studies independently and/or in groups (LO6)

able to Practicing laboratory work both independently and in groups to demonstrate the principles of plant systematics concepts (LO6a)

Plant classification methods

Topic: Flower and fruits as an object of key classification

Topic outcome:

Students are able to explain the basic concept of plant systematics

Students are able to construct the taxon of specimen samples based on the morphological characters of flowers, fruit, and seeds.

Introduction

Classification is the arrangement of entities (in this case, taxa) into some type of order. The purpose of classification is to provide a system for cataloguing and expressing relationships between these entities. Taxonomists have traditionally agreed upon a method for classifying organisms that utilize categories called ranks. These taxonomic ranks are hierarchical, meaning that each rank is inclusive of all other ranks beneath it. See the figure below for the example:

Major Taxonomic Ranks	Taxa
Kingdom	Plantae
Phylum ("**Division**" also acceptable)	Magnoliophyta
Class	Liliopsida (Monocots)
Order	Arecales
Family	Arecaceae
Genus (plural: genera)	*Cocos*
Species (plural: species)	*Cocos nucifera*

Plant classification is the science of naming organisms and placing them in a hierarchical structure, each level is given a name (e.g., kingdom, division (phylum), class, order, family, genus, species). Taxonomic units at a given level are termed taxa (singular taxon). Names of higher order taxa (e.g., kingdom, phylum, class, order, family, genus) are uninominal (i.e., each name is a single word). Names of species are binomial (e.g., Magnolia virginiana), and names of taxa below the rank of species (e.g., subspecies, varieties) are comprised of three or more words (e.g., Panicum virgatum var. cubense). Any given organism can be classified throughout the hierarchy. For example, the species sweet bay magnolia (Magnolia virginiana) is in the genus Magnolia, the family Magnoliaceae, the order Magnoliales, the class Magnoliopsida, the division Magnoliophyta, and the kingdom Plantae. Arranging scientific plant names in a hierarchical classification allows related organisms to be classified close together (e.g., all true pines are in the genus Pinus), and this assists with information retrieval.

Flower

A major diagnostic feature of angiosperms is the flower. The flower is a modified reproductive shoot, basically a stem with an apical meristem that gives rise to leaf primordia. Unlike a typical vegetative shoot, however, the flower shoot is determinate, such that the apical meristem stops growing after the floral parts have formed. At least some of the leaf primordia of a flower are modified as reproductive sporophylls (leaves bearing sporangia). Flowers are unique, differing, e.g., from the cones of gymnosperms, in that the sporophylls develop either as stamens or carpels.

The basic parts of a flower from the base to the apex are pedicle, perianth, and pistil. The pedicel is the flower stalk. (If a pedicel is absent, the flower attachment is sessile.) Flowers may be subtended by a bract, a modified, generally reduced leaf; a smaller or secondary bract, often borne on the side of a pedicel, is termed a bracteole or bractlet (also called a prophyll or prophyllum). Bracteoles, where present, are typically paired. [In some taxa, a series of bracts,

known as the epicalyx, immediately subtends the calyx (see later discussion), as in Hibiscus and other members of the Malvaceae.]

The receptacle or floral receptacle (also termed a torus, although “torus” can also be used for a compound receptacle; is the tissue or region of a flower to which the other floral parts are attached. The receptacle is typically at the very tip of the floral axis (derived from the original apical meristem). In some taxa the receptacle can grow significantly and assume an additional function. From the receptacle arise the basic floral parts. The perianth (also termed the perigonium) is the outermost, nonreproductive group of modified leaves of a flower. If the perianth is relatively undifferentiated, or if its components intergrade in form, the individual leaflike parts are termed tepals. In most flowers the perianth is differentiated into two groups. The calyx is the outermost series or whorl of modified leaves. Individual units of the calyx are sepals, which are typically green, leaflike, and function to protect the young flower. The corolla is the innermost series or whorl of modified leaves in the perianth. Individual units of the corolla are petals, which are typically colored (nongreen) and function as an attractant for pollination. Some flowers have a hypanthium (floral tube), a cuplike or tubular structure, around or atop the ovary, bearing along its margin the sepals, petals, and stamens.

Many flowers have a nectary, a specialized structure that secretes nectar. Nectaries may develop on the perianth parts, within the receptacle, on or within the androecium or gynoecium (below), or as a separate structure altogether. Some flowers have a disk, a discoid or doughnut-shaped structure arising from the receptacle. Disks can form at the outside and surround the stamens (termed an extrastaminal disk), at the base of the stamens (staminal disk), or at the inside of the stamens and/or base of the ovary (intrastaminal disk). Disks may be nectar-bearing, called a nectariferous disk.

The androecium refers to all of the male organs of a flower, collectively all the stamens. A stamen is a microsporophyll, which characteristically bears two thecae (each theca comprising a pair of microsporangia). Stamens can be leaflike (“laminar”), but typically develop as a stalklike filament, bearing the pollen-bearing anther, the latter generally equivalent to two fused thecae.

The gynoecium refers to all of the female organs of a flower, collectively all the carpels. A carpel is the unit of the gynoecium, consisting of a modified megasporophyll that encloses one or more ovules. Carpels typically develop in a conduplicate manner. A pistil is that part of the gynoecium composed of an ovary, one or more styles (which may be absent), and one or more stigmas (see later discussion). In some taxa, e.g. Aristolochiaceae and Orchidaceae, the androecium and gynoecium are fused into a common structure, known variously as a column, gynandrium, gynostegium, or gynostemium. A stalk that bears the androecium and gynoecium is an androgynophore, e.g., Passifloraceae. A stalk-like structure that bears stamens alone is termed an androphore (e.g., some Eriocaulaceae); one that bears one or more pistils is a gynophore or stipe.

Fruits

Fruits are the mature ovaries or pistils of flowering plants plus any associated accessory parts. Accessory parts are organs attached to fruit but not derived directly from the ovary or ovaries, including the bracts, axes, receptacle, compound receptacle (in multiple fruits), hypanthium, or perianth. The term pericarp (rind, in the vernacular) is used for the fruit wall, derived from the mature ovary wall. The pericarp is sometimes divisible into layers: endocarp, mesocarp, and exocarp (see fleshy fruit types, discussed later).

Fruit types are based first on fruit development. The three major fruit developments are simple (derived from a single pistil of one flower), aggregate (derived from multiple pistils of a single flower, thus having an apocarpous gynoecium), or multiple (derived from many coalescent flowers; see later discussion). In aggregate or multiple fruits, the component derived from an individual pistil is called a unit fruit. The term infructescence may be used to denote a mature inflorescence in fruit.

Seed

Aspects of seed morphology can be important systematic characters used in plant classification and identification. Some valuable aspects of seed morphology are size and shape, as well as the color and surface features of the seed coat, the outer protective covering of seed derived from the integument(s). The seed coat of angiosperms consists of two, postgenitally fused layers, an outer testa derived from the outer integument (itself sometimes divided into layers, an inner endotesta, middle mesotesta, and outer exotesta) and an inner tegmen derived from the inner integument (which can be divided into similar layers, the endotegmen, mesotegmen, and exotegmen). A seed coat that is fleshy at maturity may be termed a sarcotesta (although this may be confused with an aril, which is separate from the integuments; see later discussion). Also important in seed morphology are the shape, size, and color of the hilum, the scar of attachment of the funiculus on the seed coat, and of the raphe, a ridge on the seed coat formed from an adnate funiculus. Some seeds have an aril (adj. arillate), a fleshy outgrowth of the funiculus, raphe, or integuments (but separate from the integuments) that generally functions in animal seed dispersal. Arils may be characteristic of certain groups, such as the Sapindaceae.

Similar to the aril is a caruncle or strophiole, a fleshy outgrowth at the base of the seed; caruncles also function in animal seed dispersal, such as the carunculate seeds of Viola, violets, with regard to seed dispersal by ants.

Materials 1:

Flower and fruits of

1. Trengguli (Cassia fistula)
2. Bunga Telang (Clitoria Ternatea)
3. Lamtoro (Leucaena leucocephala)

Working procedures:

1. Write the name of the plant in the column provided on the observation sheet;
2. Write down all the morphological characteristics of the object on the observation sheet;
3. Define the family level of the specimen!;
4. Write down the classification of each specimen based on the character of the flower;
5. Then classify them based on the type of fruits.

Materials 2:

Use the reproduction structure of these specimens:

1. Pinus (Pinus merkusii): Strobilus fertil/Seed
2. Bunga Pukul Empat (Mirabilis jalapa): flower and fruit
3. Pinang (Areca catechu): Fruit
4. Red beans (Phaseolus vulgaris): Seed

Working procedures 2:

1. Observe the reproductive organs from the specimen of *Pinus merkusii* and *Mirabilis jalapa*;
2. Write your observation result in table 1;
3. Based on the reproductive organ, determine the Division of each specimen.

4. Next, carefully open the seed of Phaseolus vulgaris and the fruit of Areca;
5. Pay attention to the cotyledons, then complete table 2.

Observation result sheet: (Appendix 1)

Question:

1. The specimen of Trengguli (Cassia fistula), Bunga Telang (Clitoria Ternatea). and Lamtoro (Leucaena leucocephala) belongs to the same taxon in family Fabaceae. Describe the morphological characteristics of members of the family/tribe Fabaceae.

1. Fabaceae is divided into 3 subfamilies, namely Mimosoideae, Caesalpinioideae and Papilionoideae/Faboideaa. What is the subfamily of the bunga telang, trengguli and lamtoro? Are the three specimens belong to the same subfamily or are they different? Give an explanation.

2. Pinus sp and bunga pukul empat are included in different taxon divisions. Explain why?.

3. Name the taxon “class” of kacang merah and pinang and explain why?

Morphological characteristics and Classification of Bryophyta

Topic: 1. Morphological characteristics of Bryophyta

2. Classification of Bryophyta

Topic outcome:

1. Students can describe the morphological characters of Bryophyta
2. Students can determine the classification of Bryophyta based on their correct taxon

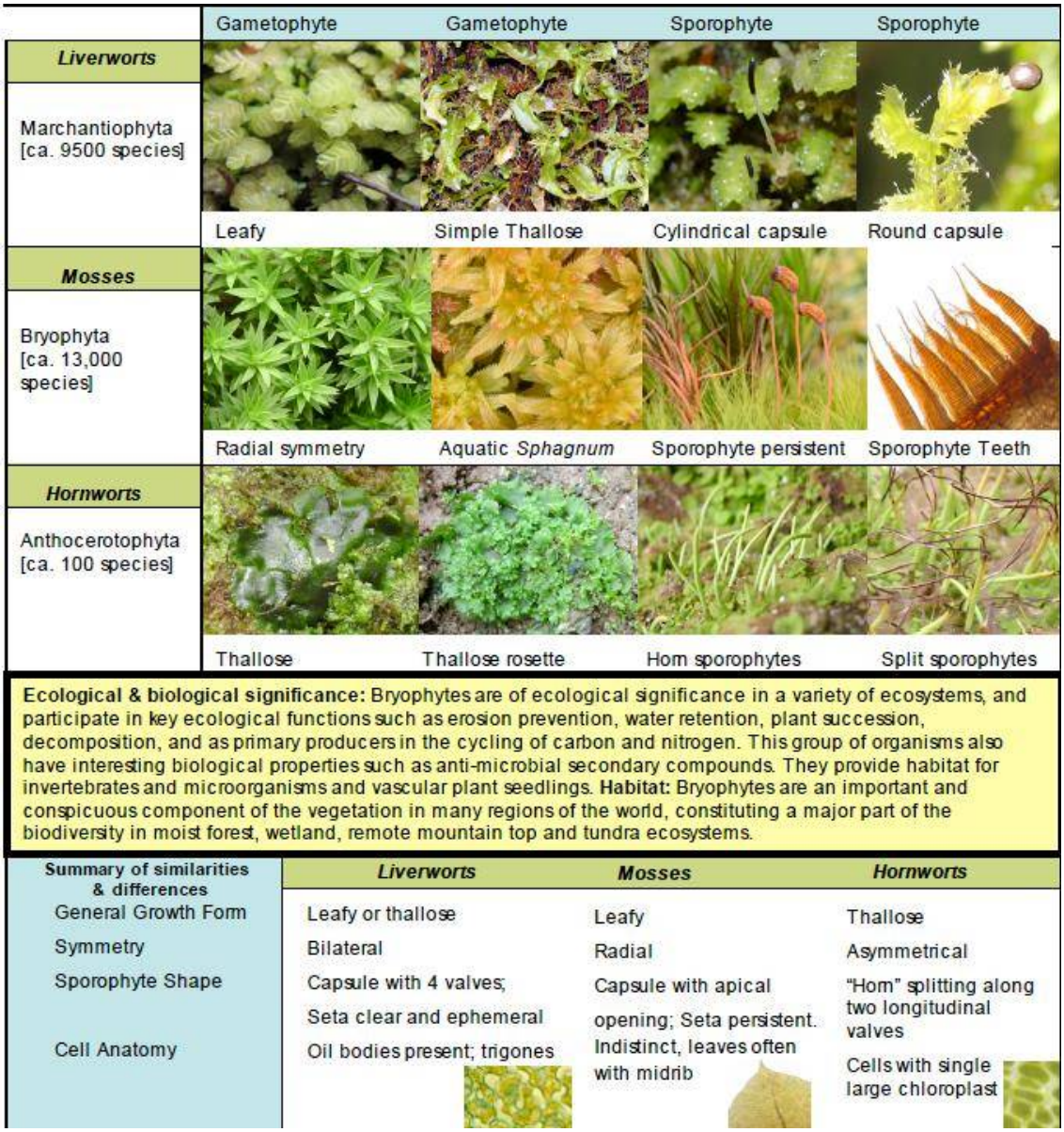
Introduction

The term ‘bryophyte’ has its origin in the Greek language, referring to plants that swell upon hydration (see Section 8.1). ‘Bryophytes’ is a generic name for plants characterized by a life cycle featuring alternating haploid and diploid generations with a dominant gametophyte (Box 1.1). In fact, bryophytes are the only land plants with a dominant, branched gametophyte, which exhibits a diversity of morphologies unparalleled in tracheophytes. This feature was long considered indicative of a unique shared ancestry, but the notion of the monophyly of bryophytes has now been strongly challenged.

Extant bryophytes belong to either liverworts (Marchantiophyta), mosses (Bryophyta in the strict sense) or hornworts (Anthocerotophyta). These lineages share several characteristics, some of which have been retained by all other land plants (e.g. an embryo which gives land plants their name ‘embryophytes’), and others that are unique (e.g. an unbranched sporophyte, with a single spore producing tissue, or sporangium). As in other extant land plants, the gametophyte lacks stomata. The three major bryophyte lineages differ from one another in a variety of attributes, most conspicuously in the architecture of the vegetative (gametophyte) body and the sporophyte, to the extent that they are easily distinguished in the field.

Liverworts, mosses, and hornworts differ from the vascular plants in lacking true vascular tissue and in having the gametophyte as the dominant, photosynthetic, persistent, and free-living phase of the life cycle. It is likely that the ancestral gametophyte of the land plants was thalloid in nature, similar to that of the hornworts and many liverworts. The sporophyte of the liverworts, mosses, and hornworts is relatively small, ephemeral, and attached to and nutritionally dependent upon the gametophyte (see later discussion).

The relationships of the liverworts, mosses, and hornworts to one another and to the vascular plants remain unclear. Many different relationships among the three lineages have been proposed, one recent of which is seen in the Figure below:



Materials:
Specimen of Bryophyta from Jember University (Politrichum, Octoblepharum, Radulina)

Equipment:
1. Luv magnification 15X
2. Microscope stereo
3. Petri disc
4. Pinset
5. Object and cover glass

Working procedures:
1. Prepare the equipment and materials;
2. Observe the available specimens, write down the classification, and then write down the morphological characteristics carefully.
3. Draw the Specimen on the Observation Sheet;
4. Find the characteristics of the specimen

Observation result sheet: (Appendix 1)

Morphological characteristics and Classification of Pteridophyta

Topic: 1. Morphological characteristics of Pteridophyta

2. Classification of Pteridophyta

Topic outcome:

1. Students can determine the classification of Pteridophyta based on thier correct taxon
2. Students are able to explain Morphological characteristics and Classification of Pteridophyta

Introduction

The word Pteridophyta is of Greek origin. Pteron means “feather” and Phyton means plant. The plants of this group have feather like fronds (leaves). The Peridophytes are assemblage of flowerless, seedless, spore bearing vascular plants that have successfully invaded the land. Pteridophytes have a long fossil history on our planet. They are known from as far back as 380 million years. Fossils of pteridophytes have been obtained from rock strata belonging to Silurian and Devonian periods of the Palaeozoic era. So the Palaeozoic era sometimes also called the “The age of pteridophyta”. The fossil Pteridophytes were herbaceous as well as arborescent. The tree ferns, giant horse tails and arborescent lycopods dominated the swampy landscapes of the ancient age. The present day lycopods are the mere relicts the Lepidodendron like fossil arborescent lycopods. Only present day ferns have nearby stature of their ancestors. Psilotum and Tmesipteris are two surviving remains of psilopsids, conserve the primitive features of the first land plants.

In the plant kingdom, pteridophytes occupy a position in between bryophytes and gymnosperms, and therefore they have some similarities with the bryophytes on the one hand and with the gymnosperms on the other hand. The similarities with bryophytes are: presence of sterile jacket around the antheridium and archegonium, requirement of water and moisture for the fertilization. While with gymnosperms are sporophytic plant body and it’s independent nature, differentiation of sporophyte into root, shoot and leaves, and presence of vascular tissues for conduction etc.

The presence of vascular elements in pteridophytes makes their grouping with gymnosperms and Angiosperms as Trachaeophyta. The reproduction by spores and similar events of life cycle place them among lower plants. The lower plants algae, fungi, bryophytes and pteridophytes were earlier grouped together as cryptogams. Bryophytes, Pteridophytes and Gymnosperms are also classified as Archegoniatae due to the presence of a common reproductive body archegonium.

Classification

Latest classification proposed by A. R. Smith (2006) and co-workers

Scientists of three different countries from USA, A.R. Smith, K.M. Preyer and P.G. Wolf (Sweden), E. Schuettpelz and H Schneider (Germany) presented a revised classification of extant ferns. They divided all vascular plants into two groups on the basis of phylogenetic studies. Recent phylogenetic studies have revealed a basal dichotomy within vascular plants, separating the lycophytes (less than 1% of extant vascular plants) from the euphyllophytes. Living euphyllophytes, in turn, comprise two major clades: the spermatophytes (seed plants), which are in excess of 260,000 species (Thorne, 2002; Scotland & Wortley, 2003), and the monilophytes (ferns, sensu Pryer& al., 2004b), with about 9,000 species, including horsetails, whisk ferns, and all eusporangiate and leptosporangiate ferns. Plants that are included in the lycophyte and fern clades (Monilophytes) are all spore-bearing or “seed-free”, and because of this common feature their members have been lumped together historically under various terms, such as “pteridophytes” and “ferns and fern allies”—paraphyletic assemblages of plants.

The focus of this reclassification is exclusively on ferns. Within ferns, they recognized four classes (Psilotopsida; Equisetopsida; Marattiopsida; Polypodiopsida), 11 orders, and 37 families.

Class 1. Psilotopsida
- A . Order Ophioglossales.
  - 1. Family Ophioglossaceae.
- B . Order Psilotales.
  - 2. Family Psilotaceae

Class 2. Equisetopsida
- C . Order Equisetales.
  - 3. Family Equisetaceae.

Class 3. Marattiopsida
- D . Order Marattales.
- 4. Family Marattiaceae .

Class 4. Polypodiopsida
- E . Order Osmundales
  - 5. Family Osmundaceae.
- F . Order Hymenophyllales.
  - 6. Family Hymenophyllaceae
- G . Order Gleicheniale.
  - 7. Family Gleicheniaceae.
  - 8. Family Dipteridaceae
  - 9. Family Matoniaceae.
- H . Order Schizaeales.
  - 10. Family Lygodiaceae.
  - 11. Family Anemiaceae
  - 12. Family Schizaeaceae.
- I . Order Salviniales
  - 13. Family Marsileaceae.
  - 14. Family Salviniaceae
- J . Order Cyatheales.
  - 15. Family Thyrsopteridaceae.
  - 16. Family Loxomataceae.
  - 17. Family Culcitaceae
  - 18. Family Plagiogyriaceae.
  - 19. Family Cibotiaceae
  - 20. Family Cyatheaceae
  - 21. Family Dicksoniaceae
  - 22. Family Metaxyaceae
- K . Order Polypodiales
  - 23. Family Lindsaeaceae
  - 24. Family Saccolomataceae
  - 25. Family Dennstaedtiaceae
  - 26. Family Pteridaceae
  - 27. Family Aspleniaceae
  - 28. Family Thelypteridaceae
  - 29. Family Woodsiaceae
  - 30. Family Blechnaceae
  - 31. Family Onocleaceae
  - 32. Family Dryopteridaceae
  - 33. Family Lomariopsidaceae

34. Family Tectariaceae
35. Family Oleandraceae
36. Family Davalliaceae
37. Family Polypodiaceae

Materials :

Specimen of Pteridophyta from Jember University (Equisetum, Psilotum, Lygodium, Selaginella, Pteris)

Equipment:

1. Luv magnification 15X
2. Microscope stereo
3. Petri disc
4. Pinset
5. Object and cover glass

Working procedures:

1. Prepare the equipment and materials;
2. Observe the available specimens, write down the classification, and then write down the morphological characteristics carefully.
3. Draw the Specimen on the Observation Sheet;
4. Find the characteristics of the specimen

Observation result sheet: (Appendix 1)

Morphological characteristics and Classification of Gymnospermae

Topic: 1. Morphological characteristics of Pinophyta

2. Classification of Pinophyta

Topic outcome:

1. Students can determine the classification of Pteridophyta based on their correct taxon

2. Students are able to explain Morphological characteristics and Classification of Pinophyta

Introduction

Recent cladistic analyses using multiple gene sequences have provided strong evidence that the Spermatophyta (seed plants) are composed of two sister groups: Gymnospermae and Angiospermae. The Gymnospermae, or gymnosperms (after gymnos, naked + sperm, seed), are called that because the ovules are not enclosed by a surrounding carpel layer (thus, being "naked") at the time of pollination. (Note that the developing seeds are often enclosed, e.g., by megasporophylls or ovuliferous scales, after pollination.) Gymnosperms are essentially nonflowering seed plants.

Knowledge of relationships within the gymnosperms is still in flux, but some phylogenetic studies show the cycads (Cycadophyta) as the most basal lineage, followed by the Ginkgo group (Ginkgophyta), then the conifers (Coniferae). Interestingly, recent analyses place the Gnetales either as sister to the conifers or within the conifers, often as the sister group to the Pinaceae. However, the precise placement of the Gnetales is still contested and needs further investigation. (See Mathews 2009.)

Cycadophyta—Cycads

The Cycadophyta (also known as Cycadales), or cycads, are a relatively ancient group of plants that were once much more common than today and served as fodder for plant-eating nonavian dinosaurs. Extant cycads are now fairly restricted in distribution, consisting of approximately 320–340 species in 11 genera. Cycads are found in southeastern North America, Mexico, Central America, some Caribbean islands, South America, eastern and southeastern Asia, Australia, and parts of Africa. Many cycads throughout the world are of economic importance in being used as a source of food starch (sometimes termed "sago"), typically collected from the apex of the trunk just prior to a flush of leaves or reproductive structures.

Some cycads, especially Cycas revoluta, the "sago palm," are planted horticulturally. Cycads are an apparently monophyletic lineage consisting of plants with a mostly short, erect stem or trunk, rarely tall and palmlike (as in the strangely named Microcycas). The trunk bears spirally arranged, mostly pinnately compound leaves. Only the genus Bowenia has bipinnately compound leaves. The trunks of cycads do not usually exhibit lateral (axillary) branching; thus, the loss of axillary branching on the aerial trunk is diagnostic for the cycads. Interestingly, cycad pinnae (Cycas) or leaves (some Zamiaceae; e.g., Bowenia) exhibit circinatevernation (Figure 5.14B) as in many ferns, perhaps a primitive retention that was lost in other seed plants. Reproductively, all cycad individuals are either male or female; this plant sex is termed dioecious.

All cycads have pollen cones or strobili (also called male cones/strobili). Recall that cones are determinate shoot systems, consisting of a single axis that bears sporophylls, modified leaves with attached sporangia. Pollen cones consist of an axis bearing microsporophylls, each of which bears numerous microsporangia. These microsporangia produce great numbers of haploid microspores, each of which develops into a pollen grain, an immature, endosporic, male gametophyte. Interestingly, the pollen of all cycads (like the Ginkgophyta,to be

discussed) release motile sperm cells within the ovule of a seed cone, a vestige of an ancestrally aquatic condition.

Recent evidence (e.g., Rai et al., 2003) suggests that cycads are best grouped as two families: Cycadaceae and Zamiaceae, differing primarily in the absence of seed cones in the former. In the Cycadaceae, seeds are produced on the margins of numerous megasporophylls, which are aggregated not in cones but at the trunk apex in dense masses. In contrast, all members of the Zamiaceae have seed [ovulate] cones or strobili (also called female cones/strobili). Seed cones (Figures 5.15C–E, 5.16C,D,F-I) consist of an axis bearing megasporophylls, each of which bears two seeds (Figure 5.16E,H,I). There is variation in the size and shape of the seed cones, megasporophylls, and seeds within groups. See Johnson and Wilson (1990c) for general information, Rai et al. (2003) and Hill et al. (2003) for a phylogenetic analyses. Cycadaceae—Cycad family (Greek koikas or kykas, for a kind of palm). 1 genus (Cycas, incl. Epicycas)/100–110 species. The Cycadaceae consist of dioecious trees to perennial herbs. The roots are often vesicular-arbuscular mycorrhizal; some adventitious roots are “coralloid,” being ageotropic (growing upward), branched and shaped like coral, and containing symbiotic, nitrogen-fi xing cyanobacteria in the outer tissues. The stem is unbranched or dichotomously branched, either an aerial trunk, covered with persistent leaf bases, or subterranean from adventitious buds, the stem apex at groundlevel. The leaves are spiral, petiolate (petiole margins with prickles), pinnately compound, evergreen, and coriaceous, forming by means of circinate vernation, in which involute leafl ets are coiled early in development; mature leafl ets have a single midvein; nonphotosynthetic, rigid cataphylls are typically produced in fl ushes alternately with photosynthetic leaves. The pollen cones are large, terminal from the trunk apex, with numerous microsporophylls, each abaxially bearing numerous, spherical microsporangia. The seed-bearing reproductive structures are not organized in determinate cones, consisting of numerous stalked, apically toothed to pinnately divided megasporophylls surrounding the trunk apex. The seeds are large, [1] 2–8, born marginally on each megasporophyll; the embryo has 2 cotyledons.

Members of the Cycadaceae are distributed in E. Africa, E. and S.E. Asia, and N. Australia. Economic importance includes cultivated ornamentals (esp. Cycas revoluta, sago-palm), food derived from the pith of the trunk (known as “sago,” made into a fl our, bread, that of some spp. toxic/carcinogenic), and edible seeds (after removal of toxins; e.g., C. media, of Australia, New Guinea). See Norstog and Nicholls (1997), Hill (1998 onwards; web site), Johnson and Wilson (1990b), and Jones (2002) for general information; Hill et al. (2004) and Walters and Osborne (2004) for classifi cation and nomenclature; and Hill et al. (2003) for a phylogenetic analysis. The Cycadaceae are readily distinguished in consisting of dioecious trees or perennial herbs, having trunks or subterranean stems, with large, coriaceous, evergreen, pinnate leaves (vernation involute circinate), and large, determinate pollen cones, the ovulate reproductive structures not organized as cones, consisting of numerous toothed to divided megasporophylls arising from apex of trunk, each bearing one or more marginal ovules/seeds.

Coniferae—Conifers

The Coniferae, or conifers (also known as Pinophyta or Coniferophyta), are an ancient group of land plants that were once dominant in most plant communities worldwide. Today, they have largely been replaced by angiosperms, but still constitute the primary biomass of various “coniferous” forests. Conifers comprise a monophyletic group of highly branched trees or shrubs with simple leaves, the latter a possible apomorphy shared with the ginkgophytes. Leaves of conifers are often linear, acicular (needle-like), or subulate (awl-shaped; see Chapter 9), although they are sometimes broad and large. In some conifers the leaves are clustered into short shoots, in which adjacent internodes are very short in length. An extreme of this is the fascicle, e.g., in species of Pinus, the pines. A fascicle is a specialized short shoot consisting of stem tissue, one or more needle-shaped leaves, and persistent basal bud scales. A second, apparent apomorphy of the conifers, including the Gnetales (discussed later), is the loss of sperm cell motility. This distinguishes the conifers from the cycads and ginkgophytes, which have flagellated sperm cells. Conifers, like all extant seed plants, have pollen tubes,

within which the male gametophytes develop. As in cycads and Ginkgo, these pollen tubes are haustorial, consuming the tissues of the nucellus (megasporangial tissue) for up to a year or so after pollination. One difference, however, (likely correlated with sperm nonmotility) is that the male gametophyte of conifers delivers the sperm cells more directly to the egg by the growth of the pollen tube to the archegonial neck, and release of nonmotile sperm cells near the egg. This type of pollen tube and sperm transfer in conifers is known as siphonogamy, as opposed to zooidogamy. (Because there is more than one archegonium per seed, multiple fertilization events may occur, resulting in multiple young embryos, but usually only one survives in the mature seed.) Reproductively, conifers produce pollen cones and seed cones, either on the same individual (monoecy) or, less commonly, on different individuals (dioecy). As with all vascular plants, cones consist of an axis that bears sporophylls. As in cycads, pollen cones consist of an axis with microsporophylls. The microsporophylls bear microsporangia, which produce pollen grains. The pollen grains of some (but not all) conifers are interesting in being bisaccate, in which two bladder-like structures develop from the pollen grain wall. These saccate structures, like air bladders, may function to transport the pollen more efficiently by wind. They may also function as flotation devices, to aid in the capture and transport of pollen grains by a pollination droplet formed in the nonflowering seed plants.

Gnetales

The Gnetales, also referred to as the Gnetopsida or Gnetophyta, are an interesting group containing three extant families: Ephedraceae (consisting solely of Ephedra, with ca. 40 species), Gnetaceae (consisting solely of Gnetum [including Vinkiella], with ca. 30 species), and the Welwitschiaceae (monospecific, consisting of Welwitschia mirabilis). The Gnetales have often been thought to be the sister group to the angiosperms, the two groups united by some obscure features, possibly including whorled, somewhat “perianth-like” microsporophylls in structures that may resemble flowers (see Chapter 6). However, as reviewed earlier, recent molecular studies have placed the Gnetales within the conifers, usually sister to the Pinaceae. Although their classification is still contested, they are placed in the Coniferae here. The Gnetales are united by (among other things) the occurrence of (1) striate pollen; and (2) vessels with porose (porelike) perforation plates, as opposed to scalariform (barlike) perforation plates in basal angiosperms. The vessels of Gnetales were derived independently from those of angiosperms. The reproductive structures in various Gnetales show some parallels to the flowers of angiosperms. Species of Gnetum of the Gnetaceae are tropical vines (rarely trees or shrubs) with opposite (decussate), simple leaves, looking like an angiosperm but, of course, lacking true flowers. Welwitschia mirabilis of the Welwitschiaceae is a strange plant native to deserts of Namibia in southwestern Africa. An underground caudex bears only two leaves, these becoming quite long and lacerated in old individuals. Pollen and seed cones are born on axes arising from the apex of the caude). Ephedra of the Ephedraceae is a rather common desert shrub and can be recognized by the photosynthetic, striate stems and the very reduced scalelike leaves, only two or three per node. Pollen or seed cones may be found in the axils of the leaves. See Kubitzki (1990a,b,c,d) for information on the Gnetales. Recently, the occurrence of a type of double fertilization was verified in species of the Gnetales. Double fertilization in Ephedra entails the fusion of each of two sperm cells from a male gametophyte with nuclei in the archegonium of the female gametophyte. One sperm fuses with the egg nucleus and the other fuses with the ventral canal nucleus. In fact, the fusion product of sperm and ventral canal cell may even divide a few times mitotically, resembling angiospermous endosperm (Chapter 6), but this does not persist. Thus, double fertilization, which has long been viewed as a defining characteristic of the angiosperms alone, was recently interpreted as a possible apomorphy of the Gnetales and angiosperms together (formerly called the “anthophytes”). This notion is rejected with the current acceptance of seed plant relationships as seen in Figure 5.1, in which the Gnetales are nested within the conifers. Thus, double fertilization in the Gnetales and angiosperms presumably evolved independently.

Classification of Gymnospermae

Cycadaceae (1/100-110)
Zamiaceae (10/220-230)
Ginkgophyta
Ginkgoaceae (1/1)
Coniferae [Pinophyta]
Pinopsida
Pinaceae (12/225)
Cupressopsida
Araucariaceae (3/32)
Cupressaceae (32/130)
Phyllocladaceae (1/5)
Podocarpaceae (17/167)
Sciadopityaceae (1/1)
Taxaceae (incl. Cephalotaxaceae) (6/28)
Gnetales
Ephedraceae (1/40)
Gnetaceae (1/30)
Welwitschiaceae (1/1)

Materials:

Specimen of Gymnospermae from Jember University (Cycas, Agathis, Araucaria, Pinus, Thuja, Gnetum)
Location and route: 1. FMIPA - KP - FKIP G3 - FKIP G1

Equipment:

1. Luv magnification 15X
2. Microscope stereo
3. Petri disc
4. Pinset
5. Object and cover glass

Working procedures:

1. Prepare the equipment and materials;
2. Observe the available specimens, write down the classification, and then write down the morphological characteristics carefully.
3. Draw the Specimen on the Observation Sheet;
4. Find the characteristics of the specimen

Observation result sheet: (Appendix 1)

Morphological characteristics and Classification of Magnoliophyta - Liliopsida

Topic: 1. Morphological characteristics of Magnoliophyta –Liliopsida

2. Classification of Magnoliophyta -Liliopsida

Topic outcome:

1. Students can determine the classification of Liliopsidaa based on thier correct taxon

2. Students are able to explain Morphological characteristics and Classification of Liliopsida

Introduction

Woody or herbaceous plants. Secretory cells with oily contents ordinarily present in the parenchymatous tissues. Vessels with scalariform or simple perforations or vessels wanting. Sieve-element plastids usually containing protein crystalloid (Pc-type) or fi laments (Pf-type), often also starch, in some families only starch (S-type). Stomata commonly paracytic. Flowers bisexual or less often unisexual, frequently spiral or spirocyclic, actinomorphic. Stamens mostly numerous. Tapetum usually secretory. Microsporo genesis successive or simultaneous. Pollen grains 2-celled or less often 3-celled, 1-colpate, 2-colpate, 3–6-colpate, rugate, porate, or often inaperturate. Gynoecium mostly apocarpous. Ovules bitegmic or much less often unitegmic, usually crassinucellate. Endosperm cellular or nuclear. Seeds mostly with small or minute embryo and copious endosperm, sometimes accompanied or largely replaced by perisperm. Cotyledons typically 2, but occasionally 3 or 4 (Degeneriaceae, Idiospermaceae). Commonly producing neolignans and/or benzyl isoquinoline alkaloids, but without ellagic acid and iridoid compounds. The subclass Magnoliidae includes a number of relatively very archaic orders and families of fl owering plants. All of them are extremely heterobathmic, that is, they have a very disharmonious combination of both primitive and derived characters. Different families of the magnoliids developed in different directions. Although all of them most probably evolved from a common ancestral stock. The basal group of fl owering plants are superorder Nymphaeanae, which include the most archaic families, beginning with Amborellaceae and ending with Ceratophyllaceae.

Materials :
Specimen of Liliopsida from Jember University (Raphis, Chrysalidocarpus, Costus, Canna, Pistia, Bambu, Caryota, Heliconia, Musa, Roystonea)
Location : FMIPA , Kantor Pusat

Equipment:
1. Luv magnification 15X
2. Microscope stereo
3. Petri disc
4. Pinset
5. Object and cover glass

Working procedures:
1. Prepare the equipment and materials;
2. Observe the available specimens, write down the classification, and then write down the morphological characteristics carefully.
3. Draw the Specimen on the Observation Sheet;
4. Find the characteristics of the specimen
5.

Observation result sheet: (Appendix 1)

Morphological characteristics and Classification of Magnoliophyta: Magnoliopsida

Topic: 1. Morphological characteristics of Magnoliopsida

2. Classification of Magnoliopsida

Topic outcome:

1. Students can determine the classification of Magnoliopsida

2. Students are able to explain Morphological characteristics and Classification of Magnoliopsida

Introduction

Embryo, when differentiated, always with one cotyledon. The cotyledon usually with two main vascular bundles. Leaf venation striate or of derived types, mostly arcuate-striate or longitudinally striate (parallel), less often palmate-striate or pinnate-striate, almost always more or less closed at the apex (the veins emerging from the leaf base usually run together again at their apices). Leaves usually not clearly divided into petiole and lamina, less often more or less differentiated, but in these cases the “petiole” and the “lamina” are not homologous to those of magnoliopsids (are of secondary origin), often with sheathing base. Leaf traces usually numerous. Prophylls (including bracteoles) usually solitary and nearly always adaxial. Vascular bundles usually without cambium or rarely with vestigial cambium only. Vascular system of the stem usually consists of many separate scattered bundles or sometimes of two or more rings of vascular bundles, and the axis mostly attains its full diameter early, after which no increase in thickness takes place; only in some groups does thickening of the axis occur by means of division and enlargement of ground parenchyma cells (so-called diffuse secondary growth), as in palms, or by means of special kind of cambium that arises in the parenchyma outside the primary vascular system, as in some herbaceous and woody Lilianae. Sieve-element plastids of P-type with several to numerous cuneate (triangular) crystalloid bodies (lacking in all magnoliopsids studied except Saruma and Asarum in Aristolochiaceae). Phloem without parenchyma. Usually without clearly differentiated bark and pith. The primary root is usually ephemeral, dries out early in the growth of the plant, and is replaced by an adventitious root system that develops from the stem or (as in grasses) directly from the hypocotyl. Ontogenetically root cap and root epidermis are of different origin. Usually herbs, but often secondarily arborescent plants (primary woody plants are absent among the monocots). Flowers usually 3-merous, sometimes 4- or 2-merous, very rarely 5-merous. Nectaries predominantly septal. Pollen grains mostly 1-colpate (sulcate) or of derived types, often 1-porate. The Liliopsida most probably originated from some very ancient vesselless herbaceous member of Magnoliopsida that had atactostelic vascular system, P-type sieve-element plastids, 3-merous fl owers, apocarpous gynoecium with laminar-diffuse (scattered) placentation, bitegmic and crassinucellate ovules (with parietal tissue between the female gametophyte) and the nucellar epidermis, and primitive 2-celled and 1-colpate pollen grains. Unfortunately there is no convincing dicotylidonous sister group to the monocotyledons. According to some authors the nearest group are nymphaeids. Some of the relatively most archaic monocots have some similarities with the nymphaeids (Hallier 1905; Schaffner 1929, 1934; Eber 1934; Takhtajan 1954, 1959, 1969, 1987; Kaul 1967; Cronquist 1968, 1981, 1988). As long ago as 1905, Hallier suggested that the Nymphaeaceae (s.1.) were the “ancestors of the whole division of monocotyledons” though later (1912) he changed his opinion. According to Arber (1920: 309), the Nymphaeaceae “descended from a stock closely related to that which gave rise to the monocotyledons.” Similar ideas have also been expressed by some other botanists. The nymphaeoids and some archaic monocots do indeed have some important characters in common. In the morphology of their gynoecia the families Butomaceae and Limnocharitaceae resemble the Cabombaceae, and in their laminar-diffuse

placentation they recall the Nymphaeaceae. There are also some other important similarities, including atactostelic vascular cylinder and especially root ontogeny (see Voronin 1964) as well as the development of female gametophytes, stomatal patterns, seed anatomy, and the arrangement of the fi rst leaves (prophylls) on lateral axes. However, the sieve-element plastids of the nymphaeids are of S-type, and they are too specialized to be considered the ancestors of monocots. It is much more probable that they evolved from some remote common ancestor that was already more or less adapted to a relatively wet (but not yet aquatic) habitat. Henslow (1911) considered the distinctive features of monocots the result of the primary adaptation to an aquatic habitat while Jeffrey (1917: 415) in his classical "Anatomy of Woody Plants" suggested an aquatic or amphibious way of life might have led to the loss of cambial activity. Henslow's hypothesis has been criticized by Sargant (1903, 1904), who concluded that many of the characteristic features of the monocots may be easier explained as having arisen as a result of adaptation to a geophilous habit. But apparently nearer to the truth was Parkin (1923: 59), who suggested "the golden mean" between the two hypotheses. He writes: "Respecting the relative merits of an aquatic or geophilous ancestry of monocotyledons, the two views may be somewhat reconciled by regarding the earliest ones as neither markedly aquatic nor extremely geophilous—in fact, marsh plants with stout rhizomes. Some of their descendants have become completely hydrophytic, others sharply geophytic, while others have returned to the arborescent habit by fresh means." Apparently the ancient common ancestor of both the Nymphaeidae and monocots was a hygrophilous or perhaps even amphibious geophyte in which geophyly arose under wet terrestrial conditions – most probably under the forest canopy or in the forest margin. But as is well known, underground storage is usually a response to a resting season, and geophytes are abundant and highly diversifi ed in areas with a pronounced resting season (see Bews 1927). Therefore, they could originate in a climate having a marked dry season (Sargant 1903; Stebbins 1974). The class Liliopsida includes 4 subclasses, 31 orders, 120 families, more than 3,000 genera, and about 65,000 species.

Materials:

Specimen of Magnoliopsida from Jember University (Trengguli, Mundu, Mengkudu, Krangkungan, Kembang Sepatu, Glodogan, Glethak, Kemuning, Si Kejut, dan Kacang Pagar)

Location: FMIPA

Equipment:

1. Luv magnification 15X
2. Microscope stereo
3. Petri disc
4. Pinset
5. Object and cover glass

Working procedures:

1. Prepare the equipment and materials;
2. Observe the available specimens, write down the classification, and then write down the morphological characteristics carefully.
3. Draw the Specimen on the Observation Sheet;
4. Find the characteristics of the specimen

Observation result sheet: (Appendix 1)

Plant description

Topic 1. Morphological characteristic observation

2. Description

Topic outcome: Students are able to describe sequentially a type of plant based on the morphological character of the plant

Introduction

A description is an analytic statement describing features that characterise the taxon in question, including macro-morphological to anatomical, biochemical, karyological and molecular aspects. A description should ideally be as thorough as possible. In the ICN glossary, a description is defined as: “a published statement of a feature or features of an individual taxon; a description (or a diagnosis) is required for valid publication of a name of a new taxon (Art. 38.1(a) and 38.3); a validating description need not be diagnostic”. The current version of the ICN is clear regarding the distinction between diagnosis and description, so that the definition of both terms does not currently seem to be a problem. The same approach is confirmed in Turland (2019: 18). It should be remarked that the ICN is focused on nomenclature and not on taxonomy, and does not aim to judge whether descriptions and diagnoses adequately represent the taxa (Nicolson, 1991). Furthermore, we highlight that the discussion presented here refers to Plant Taxonomy, not to other groups of organisms also covered by the ICN, i.e., algae and fungi.

From a historical point of view, Linnaeus (1751), in his Philosophia botanica, gave the definition of a descriptio in the Adumbratio 326 (p. 256) as follows: “Descriptio […] est totius plantae character naturalis, qui describat omnes ejusdem partes externas”, and then he gave more details on how to set up and improve a description: for Linnaeus, a descriptio is an analytic statement clearly and conceptually distinct from a diagnosis, which is a synthetic statement. More recently, Ghiselin (1997), in the glossary at the end of his book, stated that a description “enumerates the properties of things, irrespective of whether or not the properties in question are defining” and a diagnosis “enumerates properties that are useful in identification”, thus highlighting the descriptive aspect of a description, which aims at completeness, and the comparative aspect of a diagnosis, which aims at succinctness. Furthermore, a diagnosis reflects the “type method” that represents the epistemological point of contact between Taxonomy and Nomenclature (Candolle, 1867; Mayr, 1989; Witteveen, 2015, 2017, 2018). More reflections on this topic can be found in Simpson (1961), Wiley (1981) and Winston (1999).

Despite the explicit and satisfactory differentiation in the ICN, we argue that the distinction of a diagnosis and a description is not clear to many taxonomists these days, especially the younger ones. New taxa, especially new species, are often described supported only by descriptions, without a diagnosis (e.g., Berry & Galdames, 2013; Van der Maesen, 2013; Palchetti & al., 2018; Shui & al., 2019; Vaezi & al., 2019; Vladimirov & al., 2019), or other times diagnostic and descriptive information is joined under one or the other (e.g., Kuijt & Delprete, 2019). In some cases, a diagnosis is presented after a description (e.g., Arigela & al., 2019; Guzmán-Guzmán, 2019; Xiao & al., 2019), which we consider that further adds to the current state of confusion. Considering the fundamentally distinct purposes of diagnoses and descriptions (see above), we argue that it would be for the benefit of Plant Taxonomy, taxonomists and users of taxonomic classifications if both a diagnosis and a description were always provided to formalise new taxa and that, for consistency, diagnoses be presented before descriptions for each taxon.

Nevertheless, the importance of distinguishing diagnoses and descriptions goes much beyond the formalisation of new taxa. In fact, monographs and other taxonomic literature presenting morphological information should ideally present both diagnoses and descriptions for taxa. This would maximise the usefulness of those treatments, in allowing distinguishing a taxon

from its relatives (e.g., a species from its congeners) in the most succinct manner, which is achieved by means of a diagnosis, and also in informing characters of the taxon in question as thoroughly as possible, which is achieved by means of a description. Synoptic works, which normally do not present descriptions of taxa, could nevertheless provide diagnoses for the taxa treated—those diagnoses, although succinct, would have enormous usefulness for the readership in order to comprehend the species concepts and delimitations adopted by the author. Currently, the vast majority of taxonomic works being published do not provide diagnoses for taxa that are not being newly described, a situation that we hope to change with the present letter.

Traditionally, the characters used for descriptions are morphologic, but with the development of new technologies, other types of information could be used, such as, e.g., chromosome number and morphology, physiological characters, biochemical characters, and DNA molecular data (e.g., Goldstein & DeSalle, 2011; Jörger & Schrödl, 2013; Renner, 2016; Bakker, 2017; Viruel & al., 2019). It is undeniable that non-morphological information can be very useful for supporting more stable and refined taxonomic classifications (Jörger & Schrödl, 2013), offering important support to morphology (although sometimes contradicting it), and most probably will see crescent use among systematists. The use of these extra types of information is undoubtedly improving the informational content for Taxonomy and Systematics as a whole. Such integrative approaches are critically important especially for the study of species complexes and cryptic species, and constitute further support for the integration (but not substitution!) of non-morphological information to the elaboration of descriptions (Tripp & Lendemer, 2014). We acknowledge that information on micro- or nanomorphological features such as chromosomic and molecular data is not always available, but its inclusion in a description is desirable and should be done when possible.

As an illustrative example, Li & al. (2012) recently published a new fern genus, Gaga Pryer & al., presenting a description and mentioning, regarding the etymology of the new taxon, that "At nucleotide positions 598–601 in the matK gene alignment, all Gaga species have 'GAGA' […], a sequence pattern not seen at this site in any other cheilanthoid fern sampled", from which the name of the genus was dedicated to a famous American pop star. Li & al. (2012) were the first to use a nucleotide sequence from which they justified the etymology of a new genus name, but they omitted this important molecular information from the description they provided for the new genus. This very relevant molecular information could have been included in the description of the new taxon, instead in the Etymology section. Furthermore, it should be noted that the first paragraph of the description they provided for Gaga is clearly a diagnosis, which is, however, not referred to as such; this fact corroborates the prevailing view, which we highlight here, that diagnoses and descriptions are nowadays being confused by many among the scientific community.

In the case of diagnoses, however, we argue that the use of non-morphological information would undo their very purpose, i.e., to provide the most succinct and accessible means for the identification of the taxon in question. Therefore, we argue that diagnoses should use only morphological characters. The use of morphological diagnoses advocated here does not preclude that non-morphological characters be used to elaborate non-morphological diagnoses, e.g., a molecular diagnosis presenting a string of nucleotides that is unique to the taxon in question. Thus, contrary to the description, the combination of different types of characters is counterproductive for diagnoses.

The abandonment of the use of morphology for the description of new taxa and for the taxonomic classification as a whole has been suggested in some recent works (e.g., Cook & al., 2010). We feel that this would have extremely negative consequences to Taxonomy and consequently to Systematics, because most of the taxonomic novelties (especially in Plant Taxonomy) are happening in contexts where molecular works are completely unavailable. Furthermore, people working with molecular phylogeny often lack experience and knowledge

of taxonomic practices and nomenclature, and there is a well-known general trend of reduction (even extinction, in some environments) of taxonomists in research institutes, universities and even museums (Agnarsson & Kuntner, 2007; Ebach & al., 2011; Wägele & al., 2011; Sluys, 2013). The development of new techniques is increasing, not decreasing the demand for taxonomic expertise and correct specimen determinations (Will & Rubinoff, 2004; Packer & al., 2009; Taylor & Harris, 2012). In sum, abandoning morphology would bring no benefits to Plant Taxonomy and would effectively stall taxonomic advancement in the regions of the world precisely where most of the unknown biodiversity occurs. This would also have nefarious consequences for biodiversity conservation, not only because many narrowly endemic species would remain unknown to science, but also because without the use of morphology, it would become essentially impossible to recognise or determine rare and/or threatened species (Ely & al., 2017; Thomson & al., 2018).

Activity 1 Morphological characteristics

Materials : Weeds from Campus

Equipment : Luv magnification 10 – 15 X

Lokasi : in-door

Working procedures:

1. Observe the object carefully, both on the characteristics of vegetative and generative organs;
2. Write down the results of these observations on the Morphological Characteristics sheet below;

Observation result sheet: (Appendix 2)

Deskription

Working procedures:

1. Write down the Habitus of the plant concerned;
2. Next, write down all the morphological characteristics of all organs in order;
3. Write down all the morphological features. in the form of a one-paragraph narrative (description);
4. Check your description and arrange the description in sequence.

Observation result sheet: (Appendix 1)

Sample: Plant description

Habitus tree, chronic, living in the yard. Tap root system. The direction of growth is upright, branching monopodial, the shape of the stem is round, woody, hard, rough surface, gray brown color. Single leaf arrangement, imperfect, not having a midrib, small penump leaves like scales, soon fall; petiole cylindrical, thickened at the tip, 1⅓ - 2⅓ cm long, scaly tightly; elliptical leaf blade – jorong sometimes oblong – knife-like, 10 – 20 cm long, 4 – 8 cm wide, flat leaf edge, pointed tip, rounded base, pinnate leaf spines, skin-like rigidity, bare upper surface, scaly lower surface, The mother of the leaf bone clearly protrudes from the lower side to the tip of the leaf, the upper side of the leaf is shallowly grooved, the branches of the leaf

bone are oblique to the top, more or less parallel to each other. Flowers are found on somewhat old branches in a series like a fan, growing sideways, hanging, each series consists of 6-12 buds, petals numbered 5; flower stalks cylindrical, thickened at the ends, 3-8 cm long, scaly tightly; there are tamMaterials petals which are initially attached to cover the flower buds, but then divide irregularly in 2 – 4 peduncles, but usually 3, 2 – 2½ cm long, scaly outer side, short hairy inner side, tight and smooth, fall off soon; flower buds spherical or spherical in shape - ovoid, blunt or rounded ends, sometimes with a slightly pointed apex; corolla composed of 5 petals, separated, petals yellowish white, elongated round shape – spatel shape, 3½ - 5 cm long, 2-3 cm wide, short hairy outer side, glabrous inner side; the stamens are numerous in 5 bundles, each bundle facing the petals, partially attached at the base, after the flower blooms it will release, the outer side is clearly grooved, the attachment of the stamens in the bundle is only or of the length of the stamen stalk, the top is free , each bundle consists of 9-12 stamens with pollen chambers at the end; ovule hitchhiking, spherical shape – elongated or ovoid, bear 5, length 0.6 – 0.7 cm, diameter 0.4 – 0.5 cm, tightly scaly; pistil stalk 3-5 cm, short hair with a small pistil. A single true fruit consisting of several fruit leaves, has several chambers, each chamber contains several seeds. The seeds are wrapped in arillus which has thick and juicy flesh that is delicious to eat, yellow in color.

Plant Identification

Topic: 1. Morphological characteristic

2. Identification

Topic outcome: Students are able to identify a plant species to a tribal or family taxon based on morphological characters

Introduction

Plant identification implies assigning a plant to a particular taxonomic group – ultimately to the species. The identification of plant specimens is its determination of being identical with or similar to another and already known plant. Identification is the determination of a taxon as being identical with or similar to another and already known elements; the determination may or may not be arrived at with the aid of literature or by comparison with the plant of known identity. No names need to be involved in the process of identifying a plant. The naming of a plant or nomenclature is different from identification. When an unknown plant is collected from a known locality, the common practice is to refer to a book accounting for the plants of that region. This contains usually the analytical keys and descriptions.

Methods of Plant Identification:

First Method:

The first step is the determination of the families to which the unknown plant belongs. Knowing the name of the family one can turn the keys to genera for determining the generic name and then for the specific identity of the plant to the species key.

Since, for many reasons, the identity and name of the plant obtained may be incorrect, it is always safe to check the description of the plant to ensure that there is a reasonable agreement between the characters observed in the unknown plant and those given in the description of the plant presumed to be.

Second Method:

The second method is the utilization of the latest floras and check list of the particular region. These comprise usually an index to the plants known for the locality and generally provide another pertinent habit, distributional and frequency data. By the process of elimination an unknown plant can be assigned to a genera having one or more species, and identification may be completed by comparison of characters with those given in any standard work accounting of the plants of that area.

Third Method:

The third method is the identification by means of monographs or revisionary works accounting for the particular family or genus. Plant Characters before Its Identification:

Study the plant specimen to be identified in detail.

Mention the following characters:

1. Nature of specimen – herbaceous, or woody; annual or perennial.

2. Phyllotaxy and venation.

3. Inflorescence type – Capitulum (e.g. Asteraceae), Cyathium (e.g. Euphorbiaceae), Verticellaster (e.g. Lamiaceae) etc.

4. Flower and its parts – actinomorphic or zygomorphic.

5. Presence of epicalyx (e.g. Malvaceae).

6. Number of sepals and petals or tepals, their aestivation.

7. Petals free (e.g. polypetalae) or fused (e.g. gamopetalae).

8. Number of stamens and their position – antipetalous (e.g. Chenopodiaecae) alternipetalous or obdiplostemonous, (e.g. Caryophyllaceae). Staminal tube (e.g. Malvaceae).

9. Count number of carpel/carpels, style – gynobasic (e.g. Lamiaceae); stigmas.

10. Type of placentation.

Keys in Plant Identification:

A key is a device for easily identifying an unknown plant by a sequence of choices between two or more statements. A key is an artificial analytical device or arrangement where by a choice is provided between two contradictory characters resulting in the acceptance of one and the rejection of the other. Statements in the keys are based on the characters of the plants (mentioned above).

For example, a key might separate taxa using the following choices:

(1) Herbaceous versus woody if herbaceous, the woody plants are eliminated;

(2) The next choice, zygomorphic flowers versus actinomorphic, if zygomorphic, the plants with actinomorphic flowers are eliminated and so forth.

Punched card keys are used in the school, colleges etc. by the students. Punched card keys consist of cards of suitable size with names of all the taxa (all families, genera or species for which the key is meant) printed on each one of them.

Each card has a number and any one character printed near one of the corners. All the taxa showing this character are indicated by a perforation in front of their names, while those lacking this character are without any perforation.

Dichotomous Keys:

A dichotomous key presents two contrasting choices or couplet at each step. The key is designed so that one part of the couplet will be accepted and the other rejected. The first contrasting characters in each couplet are referred to as the primary key characters. These are usually the best contrasting characters. Characters following the lead are secondary key characters.

The dichotomous keys are of two types, viz., Indented key (Yoked key) and Bracketed key (Parallel key).

A dichotomous key in which the first part of a contrasting couplet is followed by all subsequent couplets; each subordinate couplet being indented one step further to the right for clarity of presentation. The indented key is the one most widely used in manuals for the identification of vascular plants. In the indented key, each of the couplets is indented a fixed distance from the left margin of the page.

Bracketed or Bracket Key or Parallel Key:

A dichotomous key in which contrasting parts of a couplet are numbered and presented together, without intervening couplets, although the brackets joining each couplet are now omitted.

Example:

The plants used in the example are common genera of the family: Ranunculaceae, viz., Clematis, Anemone, Ranunculus, Aquilegia and Delphinium.

A. Indented Key:

The first choice, with in the above genera is between “Fruit a group of achenes; flowers not spurred” and “Fruit a group of follicles; flowers spurred”, these paired statements being given the same indention.

If the latter choice is taken, the next choice, shown of the indention, is between “Flowers regular, spurs 5” and “Flowers irregular, spur ‘1’. Thus the plant in question has follicles and irregular flowers with a single spur, it must be a Delphinium.

B. Bracketed Key or Parallel Key:

In this the two couplets are always next to each other in consecutive lines on the page.

The same example of bracketed key is given below:

(i) Fruit a group of achenes; flowers unspurred (2)

(i) Fruit a group of follicles; flowers spurred …… (4)

(2) Petals absent…… (3)

(2) Petals present……. Ranunculus

(3) Sepals usually 4; involucre absent……. Clematis

(3) Sepals usually 5; involucre present…… Anemone

(4) Flowers regular; spurs 5…….. Aquilagia

(4) Flowers irregular; spur 1 ……. Delphinium

The number at the right end of a line in the bracket key indicates the next numbered pair of choices to be considered.

The keys use the most conspicuous and clear-cut characters, without special regard to those considered taxonomically the most important. For this reason the sequence of taxa is often quite artificial, and such keys are frequently termed artificial keys. Artificial key is an identification key based on convenient phenotypic characters and not indicating phylogenetic relationships.

Natural key is an identification key constructed from a natural classification and indicating the supposed evolutionary relationships of the group within the branching sequences of the key.

Comparison of Indented Key and Bracketed Key:

Indented Key:

1. Each couplet has its 2 leads indented by the same amount from the left-hand margin of the page.

2. The first couplet to be consulted is the one least indented and which has its first lead at the head of the key.

3. The next appropriate couplet to be consulted is the one with its first lead immediately below the chosen lead of the previous couplet, its leads being the next least indented pair below the latter.

Bracketed Key:

1. Each couplet has its 2 leads immediately adjacent under the same left-hand number.

2. The first couplet to be consulted stands at the head of the key next to the number 1.

3. The next appropriate couplet to be consulted is indicated by the reference number to further down the key, placed on the right-hand side of the chosen lead.

Construction of Key:

In constructing a key following techniques may be followed:

1. Key should be dichotomous.

2. The first word of each lead of the couplet should be identical. For example, if the first lead of a couplet begins with the word fruit, the second lead of the same couplet must begin with the word fruit as in example.

3. The two parts of the couplet should be made up of contradictory statements so that one part will apply and the other part will not i.e., rejected.

4. Do not use overlapping ranges or vague generalities in the couplets.

5. The couplets should be of positive statement e.g., “leaves narrow versus leaves not narrow”.

6. Use distinct and readily observable features.

7. The leads of consecutive couplets should not begin with the same word, since this may cause confusion.

8. It may be necessary to provide two sets of keys in some groups; flowering versus fruiting material, vegetative versus flowering, or staminate versus pistillate for dioecious plants.

9. Couplets of a key may be numbered or lettered, or may use some combination of lettering and numbering, or may be left blank in the case of indented keys.

Keys are traditional method of identification in taxonomy. If keys are well written with adequate specimens and carefully, then the specimen can be successfully identified. Keys, however, have several major disadvantages. The use of certain characters is required even if the character is not evident in the unknown specimen.

Activity 1: Morphological observation

Materials : Specimens of weeds from campus
Equipment : Luv magnification 10 – 15 X
Lokasi : in-door

Working procedures:
1. Observe the object carefully, both on the characteristics of vegetative and generative organs;
2. Make a small note of another important characteristic that you. get, is likely a hallmark;
3. Based on the characteristics of these organs make a description;
4. Determine the important features for identification.

Observation result sheet: (Appendix 1)

Activity 2: Identification

Working procedures:
1. Put a mark (make a small note) for important features of the vegetative organs;
2. Then focus on observing only the generative organs;
3. Draw the flowers, focusing on the special features according to your notes!;
4. Based on the identity mentioned above, then determine the taxon (tribe-generic-species);
5. Repeat for the plant types from the other Materials;
6. Pay attention to the similarities they have to estimate the kinship between them.

Observation result sheet: (Appendix 1)

Activity 3 Key Identification

Topic outcome
1. Students can explain the key identification of plants and their use
2. Students can read the key identification of plants
3. Students can create a simple key identification (Parallel Dichotomy Key).

Topic 1:Practice reading Key identification of van Steenis

Equipment: Key identification book “Flora for the student in Indonesia” by van Steenis
Working procedures:
1. Find the chapter of GOLONGAN 6
2. Follow the keys until the Apocynaceae are found;
3. Write down the number and the letter:
4. Continue to search until you find the taxon by looking for the Genus number, read the sequel and write the reading direction as in point 3 above;
5. Do the same procedure for group 8 for the family Moraceae, and group 5 for the genus of Orchidaceae.

Observation result sheet: (Appendix 1)

Activity 4: Practice creating a simple key identification “parrarel key identification”

Materials: Weeds growing around the university (10 species or more) or use these plants:
1. Peanut (Arachis hypogaea)
2. Sunflower (Helianthus annuus)
3. Bugenvil (Bougainvillea spectabilis)
4. Alamanda (Allamanda cathartica L)
5. Pinus (Pinus merkusii)
6. Si Kejut (Mimosa pudica)
7. Sirih (Piper betle)
8. Bunga pukul 4 (Mirabilis jalapa)
9. Belinjo (Gnetum gnemon)

Equipment: Luv magnification 15x

Working procedures:
1. Observe the morphological characteristics of each plant material and record them in a table.
2. Based on these morphological characteristics, make a tree diagram in the following way:
    a. Determine a distinguishing feature so that the nine (9) types of plants can be grouped into two groups. Examples of distinguishing features include habitus, root system, leaf type, leaf arrangement, etc. By using such a distinguishing feature, the specimens can be clearly grouped into two smaller groups. Put arrows to indicate different groups in descending order. Write down the difference so it doesn't get confused in the next grouping;
    b. Observe and pay attention again to the members of each group that has been divided into two groups, then determine each feature again in each group so that each group can be further divided into two smaller groups.
    c. And so on until each group has only one individual
3. Make the Dichotomy Key based on the tree diagram that has been made!
4. Make the Dichotomy Key based on the tree diagram that has been made!

Observation result sheet: (Appendix 1)

Phylogenic tree

Topic outcome: Students are able to make phylogenetic trees and explain their relationship

Introduction

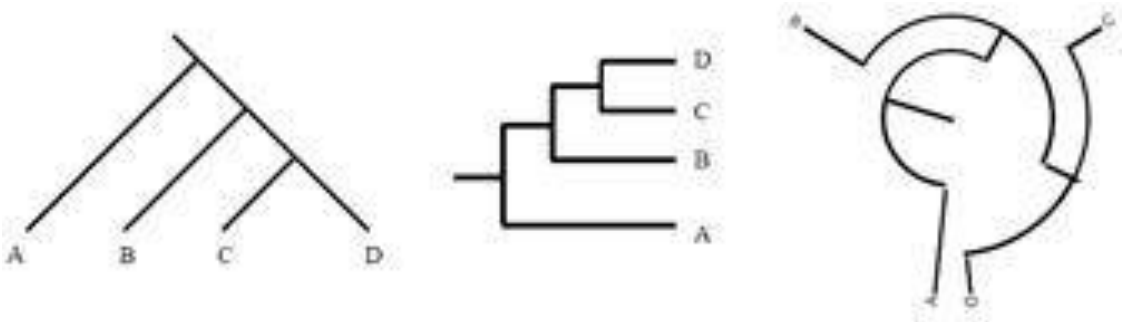
Definition of phylogenetic tree

A phylogenetic tree, also known as a phylogeny, is a diagram depicting the evolutionary lineages of various species, organisms, or genes from a common ancestor. Phylogeny is useful for organizing knowledge about biodiversity, for compiling classifications, and for providing insight into events that occurred during evolution. Furthermore, because these trees show descent from a common ancestor, and since most of the strongest evidence for evolution comes in the form of a common ancestor, one must understand phylogeny in order to fully appreciate the vast body of evidence supporting the theory of evolution.

Tree diagrams have been used in evolutionary biology since the time of Charles Darwin. Therefore, one might assume that, by now, most scientists would be very comfortable with "tree thinking" - reading and interpreting phylogeny. However, it turns out that the evolutionary tree model is somewhat counterintuitive and easily misunderstood. This may be the reason why biologists only in the last few decades have come to develop a rigorous understanding of phylogenetic trees. This understanding enables today's researchers to use phylogeny to visualize evolution, organize their knowledge of biodiversity, and structure and guide ongoing evolutionary research.

Shape of the phylogenetic tree

Trees can represent the same information but are oriented in different ways. The three trees in Figure 1, for example, have the same topology and with the same evolutionary implications. In each case, the first divergence event separated the lineage that gave rise to the A tip from the line that gave rise to the B, C, and D ends. The latter lineage then split into two lineages, one of which developed into the B end, and the other that gave rise to tips C and D. This means that C and D share a more recent ancestor with each other than with A or B. Therefore, C and D ends are more closely related to each other than A or B ends. Diagram also shows that ends B, C, and D all share a more recent common ancestor with each other than they do with ends A. Since ends B are the same distance (in terms of branch arrangement) from both C and D, we can say that B is closely related to C and D. Likewise, B, C, and D are all related to A.



Source: Baum, D. (2008). Reading a phylogenetic tree: the meaning of monophyletic groups. Nature Education, 1(1), 190.

Creating a phylogenetic tree

There are two ways to create a phylogeny tree, the first is simple (manually) and the second is by using software. In this practicum, a simple phylogenetic tree program is made based on the morphological characters of the plant specimens used. The phylogeny tree was compiled using software, namely FAMD (Fingerprint Analysis with Missing Data 1.31) and FigTree. FAMD is used to calculate variance related to finger printing data handling and analysis.

Analytical capabilities include distance-based analysis, bootstrap and consensus tree generation, allele frequency estimation, AMOVA, distance between populations, Shannon index. While FigTree is designed as a program to display phylogenetic tree graphics and as a program to generate genetic closeness numbers for publication.

Materials: Several types of plants around the campus that have been used in the practicum program for the diversity of mosses, ferns, gymnosperms, and angiosperms

The FAMD program can be accessed at the following link: http://www.famd.me.uk/famd.html

The Figtree program can be accessed at the following link: http://tree.bio.ed.ac.uk/software/figtree/

Tools: 15X magnification loupe (if necessary - bring an assistant)

Procedure:

1. Observe the specimen carefully, write down the morphological characters in the observation table.
2. Characters that appear are coded with the number 1, while characters that do not appear are marked with the number 0. For characters that are not known, they are marked with the code “N”
3. Complete the observation data by comparing all the morphological characters of the specimen and writing them down in the table.
4. Save the table in txt format using notepad.
5. Open FAMD program and import data.
6. Process the data to obtain similarity index data.
7. Save FAMD analysis data as 'outtree.ph'
8. Open the fig tree program and input the data 'outtree.ph'
9. Complete the description by activating the existing menu.
10. Good luck

Picture how it works

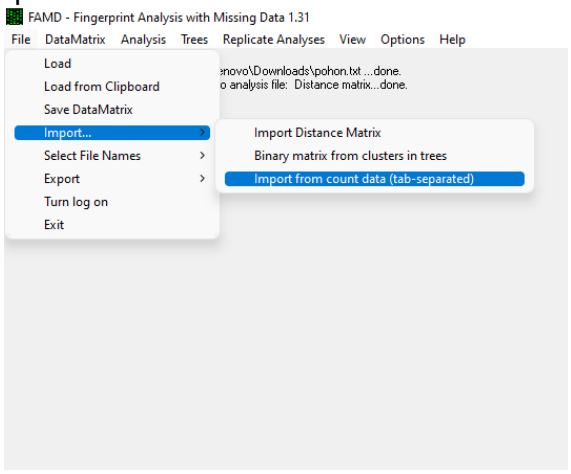
1. Input data

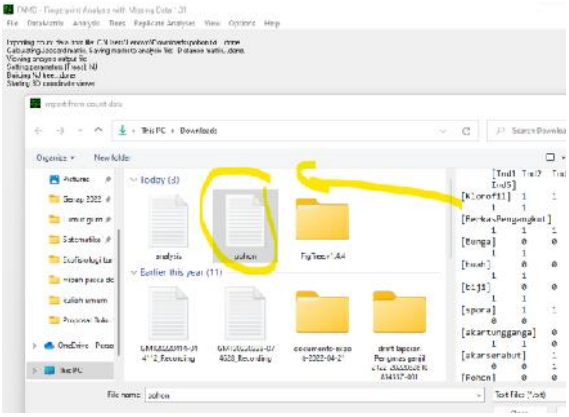
	[Ind1	Ind2	Ind3	Ind4	Ind5]
[Klorofil]	1	1	1	1	1
[BerkasPengangkut]	0	1	1	1	1
[Bunga]	0	0	1	1	1
[buah]	0	0	1	1	1
[biji]	0	0	1	1	1
[spora]	1	1	0	0	0
[akartungganga]	0	0	1	1	0
[akarserabut]	1	0	0	0	1
[Pohon]	0	0	0	1	0
[herba]	0	0	0	0	1
[semak]	1	1	1	0	0

2. Formatting “phpnklompok_x.txt”

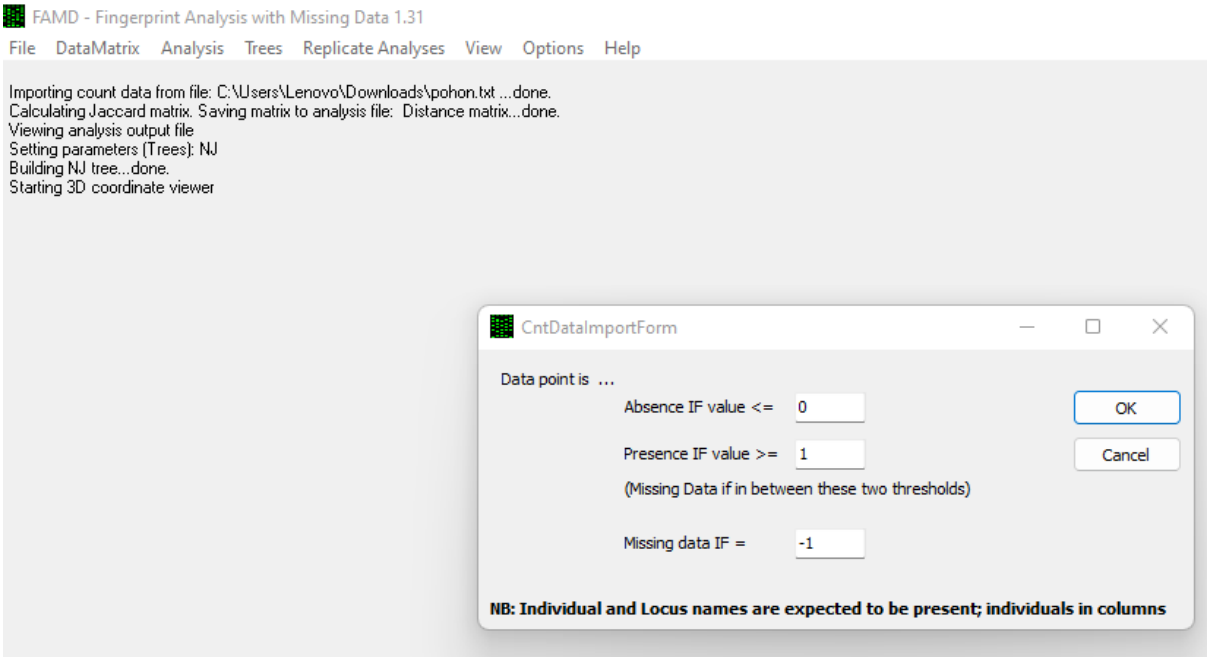
```
      [Ind1 Ind2  Ind3  Ind4  Ind5]
[Klorofil]  1     1     1     1     1
[BerkasPengangkut]      0     1     1     1     1
[Bunga]     0     0     1     1     1
[buah]      0     0     1     1     1
[biji]      0     0     1     1     1
[spora]     1     1     0     0     0
[akartungganga]   0     0     1     1     0
[akarserabut]     1     0     0     0     1
[Pohon]     0     0     0     1     0
[herba]     0     0     0     0     1
[semak]     1     1     1     0     0
```

3. Input data into FMD

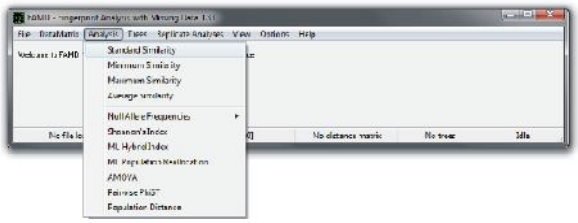


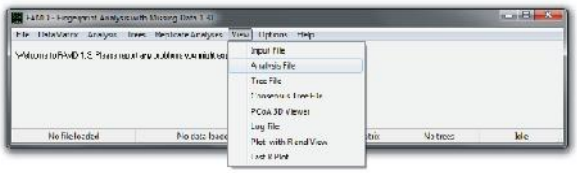


4. Define the code



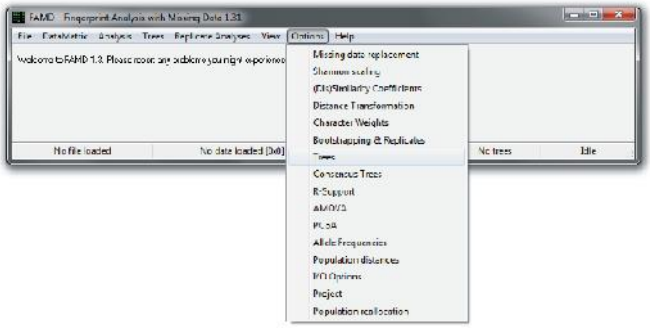
5. Process to similarity index

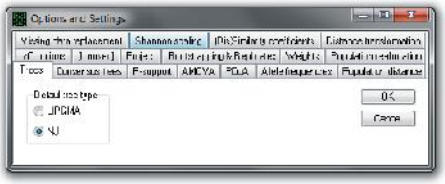




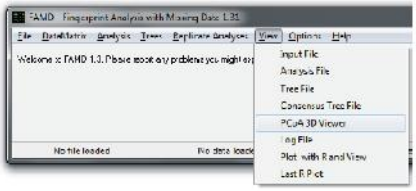
6. Set the opsi to analisis dan export

4 - set analyses options

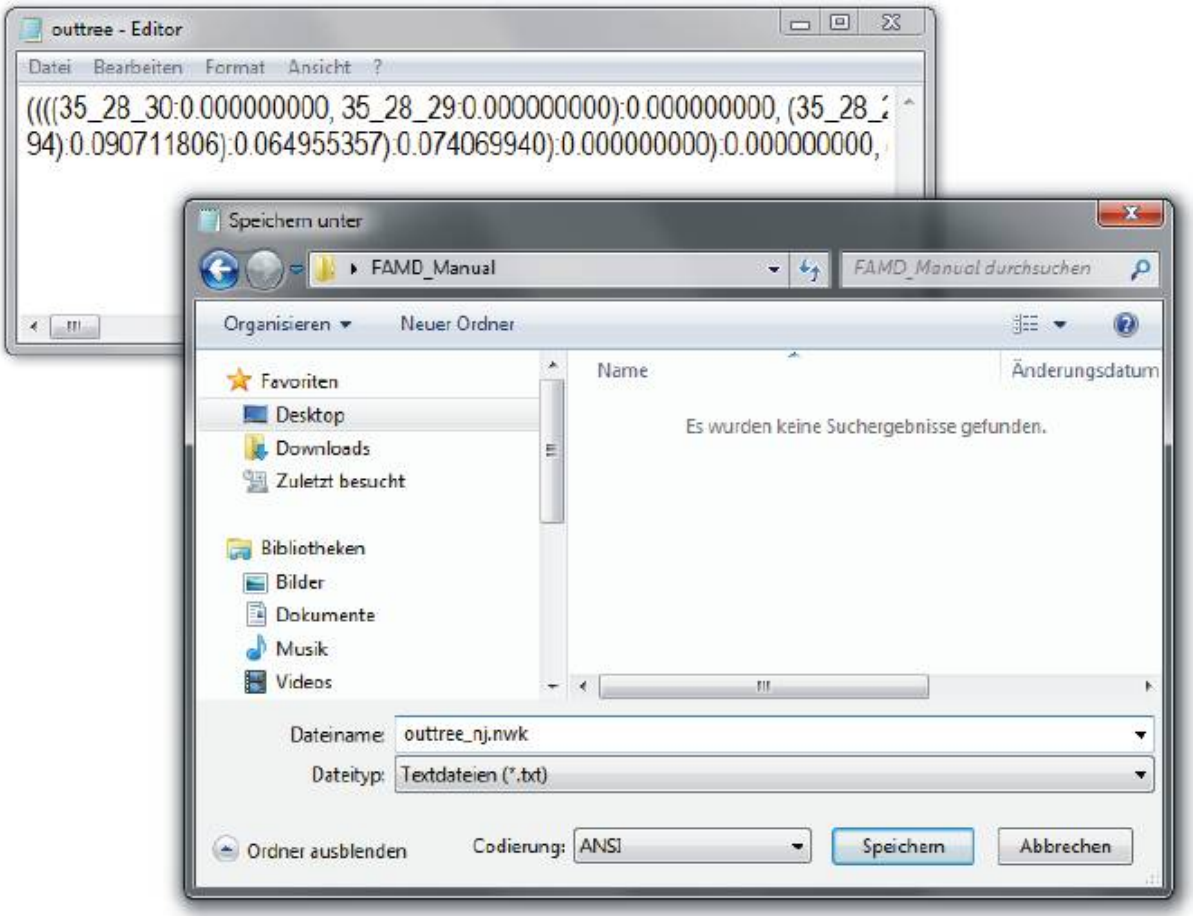




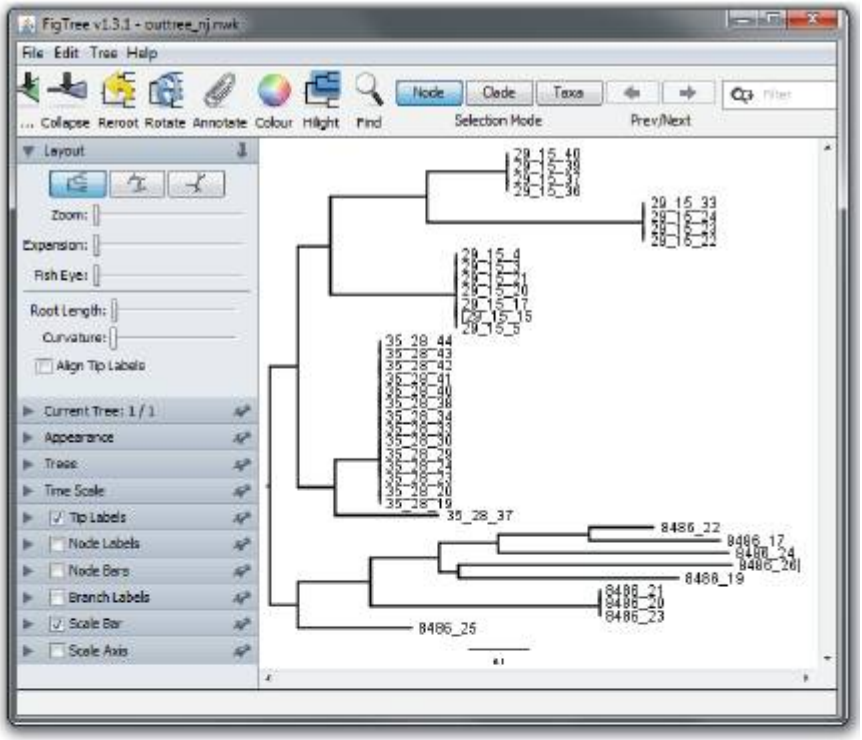
5 - compute PCoA/Neighbor-joining from the similarity matrix and view and export the results



7. Open FigTree and input data



9. Display the tree and modify by re-rooting and shape editing


FigTree v1.3.1 - outtree_nj.nwk
File Edit Tree Help
Collapse Reroot Rotate Annotate Colour Hilight Find
Node Clade Taxa
Selection Mode
Prev/Next
Layout
Zoom:
Expansion:
Fish Eye:
Root Length:
Curvature:
Align Tip Labels
Current Tree: 1 / 1
Appearance
Trees
Time Scale
Tip Labels
Node Labels
Node Bars
Branch Labels
Scale Bar
Scale Axis
29_15_33
35_28_44
35_28_37
8486_22
8486_17
8486_24
8486_20
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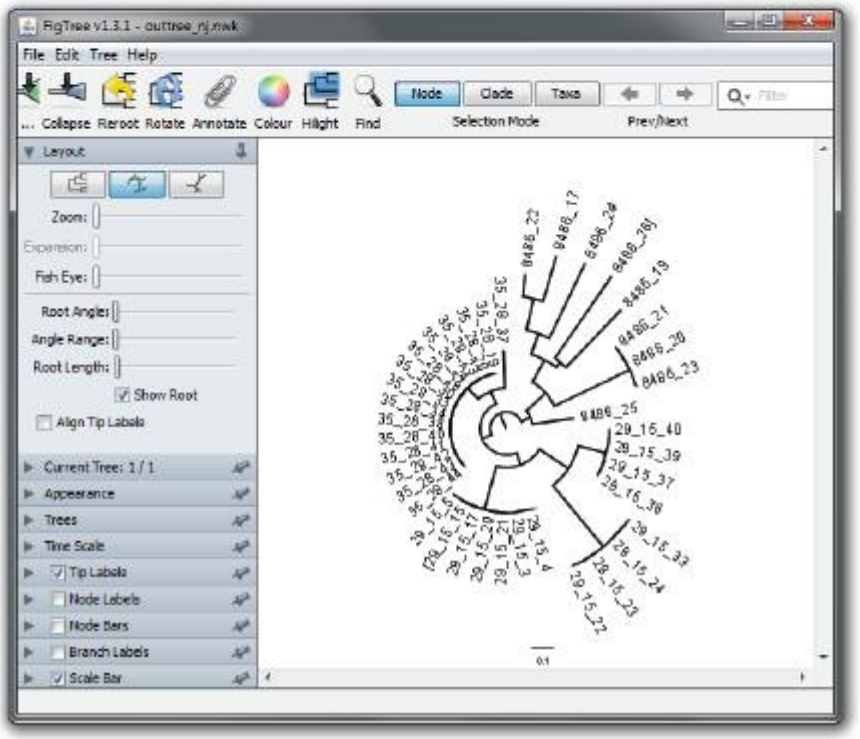
8486_23
8486_25


FigTree v1.3.1 - outtree_nj.nwk
File Edit Tree Help
Collapse Reroot Rotate Annotate Colour Hilight Find
Node Clade Taxa
Selection Mode
Prev/Next
Layout
Zoom:
Expansion:
Fish Eye:
Root Angle:
Angle Range:
Root Length:
Show Root
Align Tip Labels
Current Tree: 1 / 1
Appearance
Trees
Time Scale
Tip Labels
Node Labels
Node Bars
Branch Labels
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8486_22
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8486_23
8486_25
29_15_40
29_15_39
29_15_37
29_15_38
29_15_33
29_15_24
29_15_23
29_15_22

Field trip to Botanical Garden Eka Karya Bali

Topic outcome:

1. Students are able to describe the diversity of plants in the Eka Karya Bali Botanical Gardens

2. Students are able to verify the label of species and family of the specimen collection in the Eka Karya Bali Botanical Gardens

Introduction

Bali Botanic Garden is Indonesia's largest Botanic Garden containing the island's biggest collection of wild orchids and receiving over half-a-million visitors each year. Nestled in the refreshingly cool, mountainous region of Bedugul in central Bali, the 157-hectare Garden is an easy day trip, around 90 minute's drive north of Denpasar. The sprawling, peaceful Garden is a mix of green, open space, landscaped gardens, unique plant collections and remnant tropical forest set against the misty slopes of Tapak Hill.

Bali Botanic Garden is recognised as a leading research centre of plant biodiversity and conservation in Indonesia, conducting research in the field of horticulture, plant biodiversity and conservation with a focus on rare and endangered species from Eastern Indonesia. Seeds and plants are accepted only if their provenance is known and only if they have been collected and imported legally. Plants that have the potential for invasiveness, genetic pollution or could introduce pests or diseases are carefully screened and evaluated before acceptance. The botanic garden offers a number of scientific services and facilities in support of plant research and conservation, including a seed bank, herbarium, tissue culture laboratory, greenhouses, nursery, library and plant database. More than 2,400 species of plants can be found in the Bali Botanic Garden. This 'warehouse' of plant genetics could be very useful in future if required for restoration purposes.

Equipment : Field practical book and Camera

Working procedures:

1. All participants join a group, each group will be accompanied by an assistant.
2. Every student should read the file guide book that has been prepared by the lecturer
3. Each group will observe the types of plants found in the field based on points/sectors/tribes that have been determined in turn, namely Orchidaceae, Cactaceae, Gymnosperms, Begonoiaceae, Pteridophyta, and Arecaceae.
4. Find all living specimens according to the list!
5. Observe the specimen carefully, then draw a schematic of the habitus and possible vegetative and generative organs and write down the morphological characteristics on the Observation Sheet.
6. Find the characteristics of the specimen and write them on the worksheet;
7. Take some photos of the specimens you observe, photos that represent: habitus, stem surface, branching, leaf shape, special organs, and reproductive equipment.
8. Write the Latin name and ethnicity of each specimen listed, then confirm with IPNI and Cronquist classification (make it after the fieldwork)
9. Each group is required to submit a report in the form of a video uploaded on YouTube

Appendix 1: Observation Sheet

PLANT SYSTEMS PRACTICUM

Specimen :

Classification

Class :

Ordo :

Famili :

Genus :

Species :

<table>
<tr><td colspan="2">Morphological characteristics:</td></tr>
<tr><td colspan="2">1.</td></tr>
<tr><td colspan="2">2.</td></tr>
<tr><td colspan="2">3.</td></tr>
<tr><td colspan="2">4,
5.</td></tr>
<tr><td colspan="2"></td></tr>
<tr><td>Drawing :</td><td>Information:
1.
2.
3.
4.
dst

Description:</td></tr>
</table>

Appendix 2: Observation Sheet

Description:

References

Cronquist, A. 1981. An Integrated System of Classification of Flowering Plants. New York: Columbia University Press.

Cronquist, A. 1988. The Evolution and Classification of Flowering Plants. Second Edition. New York: Columbia University Press.

Dasuki, U.D. 1991. Sistematika Tumbuhan Tinggi, Jurusan Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Institut Teknologi Bandung, Bandung.

de Winter, W. P. and V. B. Amoroso. 2003. Plant Resources of South-East Asia Cryptogams: Fern and Fern Allies. Bogor: Prosea Foundation.

Hassemer, G., Prado, J. and Baldini, R.M., 2020. Diagnoses and descriptions in Plant Taxonomy: are we making proper use of them. Taxon, 69(1), pp.1-4.

Holttum, R. E. 1967. A Revised Flora of Malaya Volume II. Ferns of Malaya. Government Printing Office. Singapore.

Plant Identification: https://www.biologydiscussion.com/plant-taxonomy/plant-identification/an-overview-on-plant-identification/47635

Simpson, M.G., 2019. Plant systematics. Academic press.

	UNIVERSITAS JEMBER FAKULTAS MIPA PRODI BIOLOGI	KODE DOKUMEN F1.03.06

STUDENT ASSIGNMENT PLAN

SUBJECT	Plant Systematic				
CODE	MAB 1402	SKS	3-1	SEMESTER	4
TEAM TEACHING	Dra Dwi Setyati, M.Si Dr.rer.nat. Fuad Bahrul Ulum., M.Sc M.Suudi., S.Si., Ph.D				

TASK FORM

Team Project based

TASK TITLE

Diversity of Flora Eka Karya Bali botanical Garden collection

SUB CAPAIAN PEMBELAJARAN

1. Observing the morphological characteristics of collection plants in the Eka Karya Bali Botanical Gardens
2. Describe and classify selected specimens into taxon
3. Compile of phylogenetic tree of selected specimens based on their morphological character
4. Compile of systematic report on selected plants from the botanical garden collection

TASK DESCRIPTION

Research project tasks are carried out in groups using the method of direct observation of selected specimens .

TASK WORKING METHOD

1. Class divide into 6 groups.
2. Create an observation route.
3. Carry out observations, documentation and descriptions of selected specimens.
4. Perform morphological analysis and complete description.
5. Documenting specimens and editing photos.
6. Perform data tabulation
7. Complete the description of the specimen image and species description
8. Compile the classification of selected specimens.
9. Compiling a phylogenetic tree
10. Compile a report on of the result of the field work in the form of a video
11. Presenting the result of the field work in front of the class using powerpoint (PPT) media

FORM AND OUTPUT FORMAT

Object of work: Diversity of flora in Eka Karya Bali botanical garden
Output:

1. Video of the result of the field work at Eka Karya Botanical Gardens uploaded on youtube.
2. Group presentation in the form of PPT with a duration of 20 minutes per group.

INDICATORS, CRITERIA AND WEIGHT OF ASSESSMENT

Teamwork (30%)
Video (40%)
Presentation (30%)

IMPLEMENTATION SCHEDULE

1. Formation of field work committee: April 2nd week
2. Division of task groups: April 2nd week
3. Committee progress report = April 4th week and May 1 st week
4. Field work briefing = May 1st week
5. Implementation = May 2nd week

6. Data analysis = May 3rd week
7. Submission of the report on the results of the field work (video)= May 4th week
8. Presentation = June 1st week

ETC

The weight of the assignment is 25 % of the 100% assessment of this course

REFERENCES

1. Arinasa, I.B.K., Adjie, B. and Putri, D.M.S., 2017. An alphabetical list of plant species cultivated in Bali Botanic Garden. LIPI Press
2. Dharma, I.P. Dkk. 2021. Mengenal Koleksi Tematik Kebun Raya Eka Karya Bali dalam Sebuah Taman. LIPI Press
3. Stuessy, T.F., 2009. Plant taxonomy: the systematic evaluation of comparative data. Columbia University Press.

TEAMWORK ASSIGNMENT RUBRIC

Course name/Code :
Task Title :
Student name/NIM :
Table teamwork rubric :

Category	1	2	3	4	5
Working together (Individual team member's performance)	Working for at least one of this team member's roles was unacceptable	Working for one of this team member's roles is poor and the other is either poor or good	Working for one of this team member's roles is good and the other is poor	Working for one of this team member's roles is good and the other is good	Excellence work for both team member's roles
High expectation (Individual team member's performance)	This team member did not complete a majority of the work assigned to date with a grade of 60 or better.	This team member completed a majority of the work assigned to date with a grade of 60 or better.	This team member completed 75% of the work assigned to date with a grade of 60 or better.	This team member completed 90% of the work assigned to date with a grade of 60 or better.	This team member completed all of the work assigned to date with a grade of 60 or better.
Dedication and character matter (overall team performance)	A majority of the team members did NOT watch the tutorials or read the practical guideline or start the work assigned to date	A majority of the team members watched the tutorials or read the practical guideline or start the work assigned to date	75% of the team members watched the tutorials or read the practical guideline or start the work assigned to date	90% of the team members watched the tutorials or read the practical guideline or start the work assigned to date	All team members watched the tutorials or read the practical guideline or start the work assigned to date
Problem solving (overall team performance)	Team often rejects changes or constructive criticism.	Teams usually accept changes or constructive criticism.	Team often rejects changes and constructive criticism.	Team accepts changes and constructive criticism.	Team readily accepts changes and constructive criticism.

VIDEO ASSIGNMENT RUBRIC

Course Name/Code :
Group :
Student Name/NIM :

No	Indicator	1 (0-49)	2 (50-51)	3 (61-70)	4 (71-80)	5 (81-100)
1	Topic suitability k (10)					
1	Structure (Introduction, content, conclusion, closing) (15)					
	Creativity (Design, layout) (25)					
	Video quality(clarity of image, video, text audio) (20)					
	Complete information (30)					

	UNIVERSITAS JEMBER FAKULTAS MIPA PRODI BIOLOGI	KODE DOKUMEN F1.03.07

STUDENT WORKSHEETS

Lecturer : Dra Dwi Setyati, M.Si; Dr.rer.nat. Fuad Bahrul Ulum., M.Sc; M.Suudi., S.Si., Ph.D

Topic : Plant diversity of Bali Botanical Garden

Course methods : *Team Project based*

Identity

Group Number	1

Working procedure

1. All students join a group of total of 6 groups.
2. Do observation, make documentation record (photo, specimen identity) and make a short description of selected specimens.
3. Perform morphological analysis and complete description.
4. Documenting specimens and editing photos.
5. Perform data tabulation
6. Complete the description of the specimen image and species description
7. Compile the classification of selected specimens.
8. Compiling a phylogenetic tree
9. Compile a report on of the result of the field work in the form of a video
10. Presenting the result of the field work in front of the class using powerpoint (PPT) media

Result

Link video: https://www.youtube.com/watch?v=2gAuKqNvS8o

Group 1

Lembar Pengamatan

Spesimen :

Klasifikasi
Kelas :
Bangsa :
Suku/ Group :
Marga :
Jenis : _____ _____

Ciri-ciri morfologi:	
1. 2. 3. 4. 5.	
Gambar :	Keterangan:

Ciri khas :

Module designation	*: **Aquatic Ecology***
Semester(s) in which the module is taught	*: even/IV*
Person responsible for the module	1. *Dr. Dra. Retno Wimbaningrum, MSi* 2. *Rendy Setiawan, SSi., MSi.* 3. *Arif Mohammad Siddiq, SSi., MSi.* 4. *Dra Hari Sulistiyowati, MSc., PhD.*
Language	: *Bilingual*
Relation to curriculum	: *Compulsory /~~elective~~ / ~~specialisation~~* *Names of other study programmes with which the module is shared*
Teaching methods	*: Lecture- Discussion, Project/Laboratory/Field Work, Presentation,* *e.g. lecture, lesson, lab works, project, seminar etc.*
Workload (incl. contact hours, self-study hours)	*(Estimated) Total workload:* 136 hr *Contact hours (please specify whether lecture, exercise, laboratory session, etc.):* a. *Lecture- Discussion*: *56.67hr* b. *Laboratory works: 45.33hr* c. *Presentation: 34 hr* *Private study including examination preparation, specified in hours[1]:*

[1] When calculating contact time, each contact hour is counted as a full hour because the organisation of the schedule, moving from room to room, and individual questions to lecturers after the class, all mean that about 60 minutes should be counted.

Credit points	*: 3 credits or 4.53 ECTS*
Required and recommended prerequisites for joining the module	*: Terrestrial Ecology* *E.g. existing competences in ...*
Module objectives/intended learning outcomes	*Knowledge: able to analyse the principles of biology, mathematics, and other relevant natural sciences (LO 2)* ● *Analyzing the basic concepts of bioinformatics for biological data analysis (CLO 2.b)* *Skill: able to **implement** scientific methods for the management of biological resources in tropics (LO 4)* ● ***Implementing** scientific methods for the biology conservation in tropics (CLO 4.a)* *Skills: able to implement biological concepts in laboratory work and/or field studies independently and/or in groups (LO 6)* ● ***Practicing** laboratory and field works related to aquatic ecology (CLO 6.a)* ● *Using software applications and/or basic instruments for sampling and analysis in aquatic ecology (CLO 6.b)*
Content	*This course covers components (chemicals, physical, and biological) and characteristics of freshwater (lotic and lentic) , estuarine, and marine (intertidal, subtidal, and deep sea) ecosystems. **Laboratory works** cover: Equipments handling of aquatic ecology, Sampling technique and ecological data analysis*

	of physics, chemical, and biological characteristics of Bedadung River (lotic ecosystem), Ranu Klakah Lake (lentic ecosystem), and Intertidal ecosystem at Baluran National Park by ***Using basic instrument sampling. It also implements*** *ecological analysis by using* ***Microsoft Excel, Primer Software, and R Statistics*** *to determine diversity index, dominance index, species composition and correlation between biota and its environment in aquatic ecosystems* *The description of the contents should clearly indicate the weighting of the content and the level.*
Examination forms	1. *Attitude observation (5%)* 2. *Essay test* (30%) 3. *Topic presentation* (15%) 4. Pre/post test (5%) 5. Responsi (15%) 6. Activity observation (5%) 7. Equipment/software observation (10%) 8. *Report presentation of laboratory work (15%)* *e.g. oral presentation, essay, etc.*
Study and examination requirements	*: passing grade 70%* *Requirements for successfully passing the module*
Reading list	1. *Allan, J.D. & Castillo, M.M. 2007. Stream ecology: structure and function of running water. 2nd Ed. Springer. Netherlands.* 2. *Barnes, R.S.K. and Mann, K.H. 1991. Fundamental of aquatic ecology. Blackwell Scientific Publications. London.*

	3. *Brönmark, C. And Hansson, L-A. 2005. The biology of lakes and ponds. 2nd Ed. Oxford University Press. Oxford.* 4. *Clesceri, L. S., Greenberg, A. E. & Eaton, A.D. 1998. Standard methods for the examination of water and wastewater. 20th Ed. American Public Health Association, American Water Works Association, and Water Environment Federation. Washington.* 5. *Closs, G., Downes, B., and Boulton, A. 2004. A scientific introduction to freshwater ecology. Blackwell Scientific Ltd. Oxford.* 6. *Edmondson, W.T. 1959. Freshwater biology. Second Ed. John Wiley and Sons Inc. New York.* 7. *Goldsmith, F.B. and Duffey, E. 1997. Conservation management of freshwater habitats. Chapman & Hall. London.* 8. *Hauer, F.R. & Lamberti, G.A. 1996. Methods in stream ecology. Academic Press. California.* 9. *Hemminga and Duarte, C.M. 2000. Seagrass ecology. Cambridge University Press. Cambridge.* 10. *Odum, T.E. 1993. Fundamental ecology. Gadjah Mada University Press. Yogyakarta.* 11. *Related Scientific Article Journals or Web Science* *Names of textbooks, articles, etc.*

Credits to ECTS conversion formula 1 SKS TM = 50min T+60min TS+60min M (170 minutes) x 16 weeks = 45.33 Hours 1 SKS Practice = 170 min. 1 ECTS = 29.99 hours 1 Credit = 1.51 ECTS

	UNIVERSITY OF JEMBER FACULTY OF MATHEMATICS AND SCIENCE BACHELOR BIOLOGY	DOCUMENT CODE FORM PP-05

STUDENT WORKSHEET PLAN

Lecture : Rendy Setiawan, S.Si. M.Si.
Subject : Subtidal & Deep Sea Ecosystems
Teaching Model : Interactive Discussion

STUDENT IDENTITY

Name	Jocelin Andiana
Student ID	191810401034
Meet	14
Day/Date	Thursday/15 May 2021
Score	90

DISCUSSION MATERIAL

Based on your understanding of the discussion on subtidal and deep sea ecosystems, please answer these questions briefly and clearly within 60 minutes:

1. Explain why seaweed (kelp) is able to grow well in subtidal ecosystem areas?
2. Why are coral reef ecosystems very vulnerable to bleaching due to rising sea water temperatures?
3. State the benefits of hydrothermal vents for life around it?
4. Explain how the role of the deep sea for humans in general?
5. Why is there no seagrass ecosystem found in the deep sea area?

discussion results

Write the results of the discussion in this section!

1. Kelp is able to grow optimally in subtidal ecosystems because of the abundance of nutrients from sunlight for the photosynthesis process.
2. because temperature affects the metabolic processes of coral reefs. if the temperature increases, the metabolic ability of coral reefs becomes greater so that the energy expended is greater.
3. the benefit of marine hydrothermal vents is to provide nutrients in the form of chemicals needed by organisms as a food source.
4. The deep sea provides a food source in the form of fish and mollusks which are abundant in the ocean. In addition, the deep sea also maintains natural ecosystems that are still untouched by humans.
5. Seagrass requires sunlight for photosynthesis, while the deep sea does not have sunlight

	UNIVERSITY OF JEMBER FACULTY OF MATHEMATICS AND SCIENCE BACHELOR BIOLOGY	DOCUMENT CODE FORM PP-05

STUDENT WORKSHEET PLAN

Lecture : Rendy Setiawan, S.Si. M.Si.
Subject : Subtidal & Deep Sea Ecosystems
Teaching Model : Interactive Discussion

STUDENT IDENTITY

Name	Muhammad Afriyanto
Student ID	191810401035
Meet	14
Day/Date	Thursday/15 May 2021
Score	85

DISCUSSION MATERIAL

Based on your understanding of the discussion on subtidal and deep sea ecosystems, please answer these questions briefly and clearly within 60 minutes:

1. Explain why seaweed (kelp) is able to grow well in subtidal ecosystem areas?
2. Why are coral reef ecosystems very vulnerable to bleaching due to rising sea water temperatures?
3. State the benefits of hydrothermal vents for life around it?
4. Explain how the role of the deep sea for humans in general?
5. Why is there no seagrass ecosystem found in the deep sea area?

discussion results

Write the results of the discussion in this section!

1. Kelp is able to grow optimally in subtidal ecosystems because of the abundance of nutrients from sunlight for the photosynthesis process.
2. because temperature affects the metabolic processes of coral reefs. if the temperature increases, the metabolic ability of coral reefs becomes greater so that the energy expended is greater.
3. the benefit of marine hydrothermal vents is to provide nutrients in the form of chemicals needed by organisms as a food source.
4. The deep sea provides a food source in the form of fish and mollusks which are abundant in the ocean. In addition, the deep sea also maintains natural ecosystems that are still untouched by humans.
5. Seagrass requires sunlight for photosynthesis, while the deep sea does not have sunlight

	UNIVERSITY OF JEMBER FACULTY OF MATHEMATICS AND SCIENCE BACHELOR BIOLOGY	DOCUMENT CODE FORM PP-05

STUDENT WORKSHEET PLAN

Lecture : Rendy Setiawan, S.Si. M.Si.
Subject : Subtidal & Deep Sea Ecosystems
Teaching Model : Interactive Discussion

STUDENT IDENTITY

Name	Widayanti
Student ID	191810401072
Meet	14
Day/Date	Thursday/15 May 2021
Score	85

DISCUSSION MATERIAL

Based on your understanding of the discussion on subtidal and deep sea ecosystems, please answer these questions briefly and clearly within 60 minutes:

1. Explain why seaweed (kelp) is able to grow well in subtidal ecosystem areas?
2. Why are coral reef ecosystems very vulnerable to bleaching due to rising sea water temperatures?
3. State the benefits of hydrothermal vents for life around it?
4. Explain how the role of the deep sea for humans in general?
5. Why is there no seagrass ecosystem found in the deep sea area?

discussion results

Write the results of the discussion in this section!

1. Kelp is able to grow optimally in subtidal ecosystems because of the abundance of nutrients from sunlight for the photosynthesis process.
2. because temperature affects the metabolic processes of coral reefs. if the temperature increases, the metabolic ability of coral reefs becomes greater so that the energy expended is greater.
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5. Seagrass requires sunlight for photosynthesis, while the deep sea does not have sunlight

DISCUSSION PARTICIPATIF RUBRIC

Course Name :
Student Name/NIM :

	Unacceptable (<60%)	Acceptable (60-69%)	Developing (70%-79%)	Proficient (80%-89%)	Exemplary (90%- 100%)
Frequency of participation in class	Student does not initiate contribution & needs instructor to solicit input	Student initiates contribution at least in half of the recitations	Student initiates contribution once in each recitation	Student initiates contribution twice in each recitation	Student initiates contributions more than twice in each recitation
Quality of comments	Comments are uninformative, lacking in appropriate terminology. Heavy reliance on opinion & personal taste, e.g., "I love it", "I hate it", "It's bad" etc.	Comments are sometimes constructive, with no signs of insight	Comments are sometimes constructive, with occasional signs of insight Student does not use appropriate terminology; comments not always relevant to the discussion	Comments mostly insightful & constructive; mostly uses appropriate terminology Occasionally comments are too general or not relevant to the discussion	Comments always insightful & constructive; uses appropriate terminology Comments balanced between general impressions, opinions & specific, thoughtful criticisms or contributions
Listening Skills	Does not listen to others; regularly talks while others speak or does not pay attention while others speak; detracts from discussion; sleeps, etc.	Student is often inattentive and needs reminder of focus of class. Frequently makes disruptive comments while others are speaking	Student is often inattentive and needs reminder of focus of class. Occasionally makes disruptive comments while others are speaking	Student is mostly attentive when others present ideas, materials, as indicated by comments that reflect & build on others' remarks. Occasionally needs encouragement or reminder from T.A of focus of comment.	Student listens attentively when others present materials, perspectives, as indicated by comments that build on others' remarks, i.e., student hears what others say & contributes to the dialogue.