FOOD MICROBIOLOGY PRACTICUM GUIDE BOOK

1ST EDITION , 2022

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UNDERGRADUATE OF NUTRITION PROGRAM
FACULTY OF PUBLIC HEALTH UNIVERSITAS AIRLANGGA

COURSE LEARNING PLAN

FOOD MICROBIOLOGY PRACTICUM

Weeks	Date	Meetings	Study Materials	Lecture
1		1	- (already)	SRN
2		2	- (already)	SRN
3		3	-	DOM
4	9/8/2022	4	Practicum Contract Event I: Explanation of laboratory equipment and laboratory safety	DOM
5	9/15/2022	5		ALF
6	9/22/2022	6	Event II: Observing the morphology of molds and fungi	ALF
7	9/29/2022	7		ALF
8	10/20/2022	8	Event III : Observing yeast dan mildew morphology	ALF
9	27/10/2022	9		DOM
10	11/03/2022	10	Event IV : Staining of gram + and gram - bacteria	DOM
11	11/10/2022	11		DOM
12	11/17/2022	12	Event V : Observing growth microbiology	DOM
13	11/24/2022	13	Acara VI : Observing growth microbiology morphology	DOM
14	12/1/2022	14	- Practicum Examination Food Microbiology-	SRN

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PRACTICUM RULES

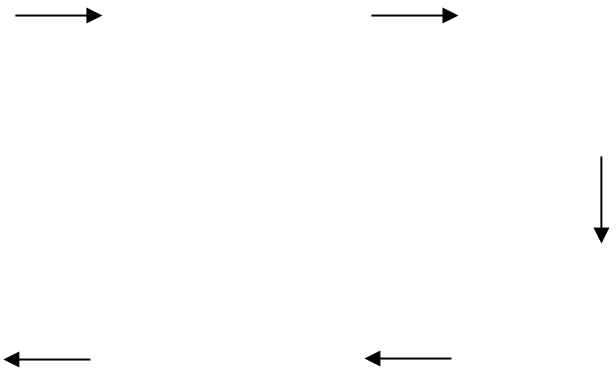
1. Students must arrive 15 minutes before the practicum schedule begins
2. Students are required to wear clean lab coats, nametags, safety equipment such as gloves and masks
3. Bags and other things that are not needed must be placed in the place provided. Students are not allowed to place items that are not needed in the practicum process on the laboratory table
4. Students work together in one table based on their assigned group
5. Each group is responsible for keeping their particular workstations tidy
6. Students must maintain the purity of the materials used and keep away from all kinds of contaminants that can interfere with control and results
7. Students must report to the laboratory if they find/do unexpected things outside the practicum protocol.

STANDARD LABORATORY OPERATING PROCEDURE

1. Clean the laboratory table with disinfectant before and after laboratory activities.
2. Wash your hands with soap and water before and after laboratory activities.
3. Do not smoke, eat or drink in the laboratory room.
4. Keep your hands away from your mouth, nose, eyes and ears while you are working in the laboratory.
5. You are not allowed to take any sample out of the laboratory room.
6. If you are contaminated or injured, call an instructor.
7. When the same pipette needs to be used more than one time, do not place it directly on the table between uses, but place it on the pipette support that has been provided

WORK PROCEDURE

(Work procedure flowchart)



REPORT WRITING PROVISIONS

1. The report is written by hand
2. The report is written in an A5 sized lined book
3. The report contains work procedures in the form of pictures and brief writing made in a flowchart
4. Reports are submitted no later than D + 6 after practicum time

REPORT FORMAT

REPORT
(PRACTICUM TITLE)

Title :
Assistant Name:
Day/Date:
Destination:

Experiment Methodology :

A. Tool
B. Material
C. Way of work
D. Discussion
E. Conclusion
F. Bibliography

REPORT COVER FORMAT

Nutrition Program
Faculty of Public Health
Universitas Airlangga

FOOD MICROBIOLOGY PRACTICUM REPORT

Student Name : ……..
NIM : ……..
Group : ……..

EVENT I – Introduction to Laboratory Equipment

Literature Review

The implementation of practicum and research activities in the microbiology laboratory goes well if laboratory users understand the equipment used for work. There are several tools that are often used in microbiology laboratories, including:

1. Laminar air flow (LAF)
   A tool as a room for aseptic work. The principle of aseptic in a room based on the outflow of air with air contamination can be minimized..

Figure 1. Laminar Air Flow

2. Mikroskop
   A tool used to see objects or micro-organisms that cannot be seen with the naked eye.

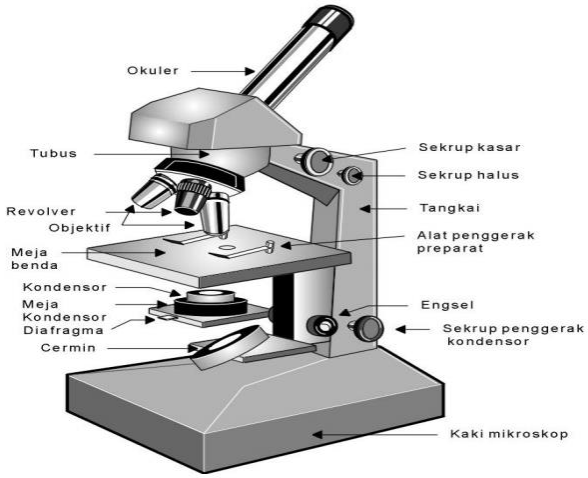


Figure 2. Parts of a Microscope

3. Autoclave
   A tool that is almost similar to a presto, this tool serves to sterilize tools and materials to be used for microbiology work.

Figure 3. Autoclave

4. Hot Plate and magnetic stirrer
   A tool used to heat media or other materials to be used in microbiological practice and to homogenize a solution with stirring..

Figure 4. Hotplate and magnetic stirrer

5. Vortex
   A tool that serves to homogenize the sample suspension

Figure 5. Vortex

6. Incubators
   A tool that functions to grow the microorganism you want to grow (for incubate).

Figure 6. Incubator

7. Drying Oven
   Tool used to dry equipment after autoclaving or dry materials.

Figure 7. Oven

8. Colony Counter
   A tool that works to count the number of colonies growing in a petri dish.

Figure 8. Colony Counter

9. pH meter
   An instrument for measuring the acidity of a solution or mixture.

Figure 9. pH meter

10. Spectrophotometer
    An instrument for measuring the concentration of a solution/mixture..

Figure 10. Spectrophotometer

11. Analytical Scales
    A tool that functions to weigh materials to be used in practicum with a high level of accuracy.

Figure 11. Analytical Scales

12. Ose Needle
    A tool used to inoculate microbes that will be transferred to another medium and to take solid media.

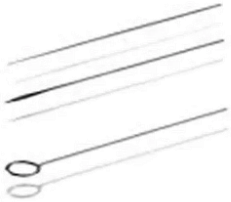


Figure 12. Ose Needle

13. Micropipette
    A tool that functions to move liquids that are quite small in volume, usually less than 1000 µl.

Figure 13. Micropipette

14. Bunsen

A tool that functions for sterilization by heating.

Figure 14. Bunsen

15. Spreader

A tool used to spread bacterial cultures contained in culture containers

Figure 15. Spreader

16. Centrifuge

Serves to precipitate and separate solids from solution, Effective in removing suspended particles that are too small to be filtered.

Figure 16. Mini Centrifuge

Objective:

1. Find out the tools used in the Microbiology Laboratory
2. Find out the form and function of glassware and laboratory equipment
3. Find out the different types of sterilization

Experiment Methodology:

A. Tools : - listed in the material
B. Ingredients : - none
C. Discussion :
D. Pictures of each tool (not photos) accompanied by an explanation of the function and how to use each tool.
E. Conclusion
F. Bibliography

EVENT 2 – Identification of Fungi by Observing Macroscopic and Microscopic Morphology

Literature Review

Mold growth on the surface of food ingredients is easy to recognize because it often forms filamentous colonies such as cotton. The body of the fungus is in the form of threads called hyphae, a group of hyphae is called mycelium. Mycelium can contain pigments with red, purple, yellow, brown, gray and so on. The fungus also forms green, blue-green, yellow, orange, pink and so on spores. Fungi are generally obligate aerobes, growth pH ranges from 2-9, growth temperature ranges from 10-35ºC, water activity (aw) 0.85 or below (ref).

Identification of fungi can be done by observing their morphology both macroscopically and microscopically. Observation of macroscopic morphology is a test carried out with the naked eye or with the help of a magnifying glass for the microorganisms to be detected. Meanwhile, microscopic observations were made to see morphology that could not be caught with the naked eye. In general, identification of fungi can be done macroscopically by observing the colonies that form. Characteristics observed may include color, colony texture, and number of colonies. Colony texture can be divided into two types, namely raised (mycelium grows towards the top of the media surface) and pressed (mycelium grows horizontally on the media). However, to find out the parts of the fungus that grows, microscopic observations are needed with the help of a microscope. The optimal magnification for observing mushroom parts is 10 x 40. Microscopic observation of mushroom morphology can also be done by staining to distinguish the parts. Lactophenol Cotton Blue (LPCB) is a reagent that is widely used as a stain for mold. Lactophenol Cotton Blue reagent contains phenol crystals, cotton blue, lactic acid, glycerol and distilled water.

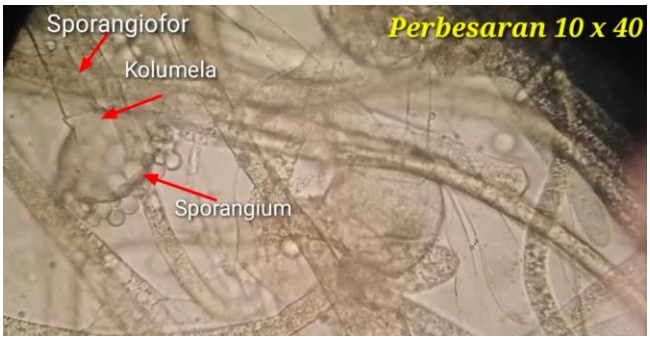


Figure 2.1 Appearance of the fungus *Rhizopus sp*. under a microscope magnification 10 x 40 (Source: Google picture)

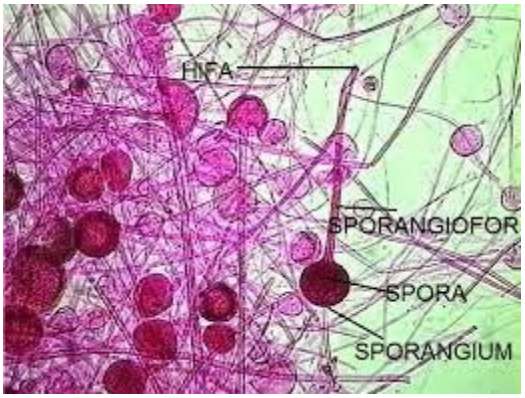


Figure 2.2 Morphology of the fungus *Rhizopus sp*. with staining (Source: Google picture)

Objective :

1. Develop the skills of transferring molds from samples to preparations.
2. Observing Rhizopus molds preparations and studying their morphology.

Experiment Methodology:

A. Tool :
Microscope
Preparation page
Cover glass

B. Ingredients:
Mold preparations

C. Ways of work

1. Cleaning glasses with tissues
2. Drops of glass preparations with aquadest in the middle
3. Taking a small amount of mold culture with a preparation needle
4. Laying on a glass preparation that has been dripped with aquadest
5. Separation with 2 preparation needles if the mycelia collects
6. Observation weak magnification first
7. Observation with moderate magnification
8. Observation of the desired parts with a strong magnification
9. Depiction of parts and provision of information.

D. Discussion:
Points to discuss:

1. Mold characteristics
2. Use of mold
3. Explanation of mold morphology
4. Comparison of the results of observations (in pictures not photos) and standard mold morphology, state the factors and reasons
5. Comparison of the results of observations and the results of observations of other groups (photos), state the factors and reasons

E. Conclusion

F. Bibliography

NOTES :

Table 2.1. Group Turn Distribution: (Food Microbiology Laboratory 3rd floor)

Turn	Group	Time	Sample
1-3a	1,2	09.00 – 09.45	Tempeh (wrapped in leaved)
2 -3a	3,4	09.45 – 10.30	Tempeh (wrapped in plastic)
3 -3a	5,6	10.30 – 11.15	Tempeh menjes
1-3b	1,2	13.00 – 13.45	Tempeh (wrapped in leaved)
2 -3b	3,4	13.45 – 14.30	Tempeh (wrapped in plastic)
3 -3b	5,6	14.30 – 15.15	Tempeh menjes

*each group brings its own sample

EVENT 3 – Detection of Yeast by Observing Macroscopic and Microscopic Morphology and Comparing with Rhizopus sp., Aspergilus sp., and Penicillium sp.

Literature Review

Yeast or yeast is a unicellular fungal microorganism which has high resistance to antibiotics, has antimicrobial properties, and has resistance to salt, acid and sugar. Yeast has the ability to produce several organic acids, such as hexanoic, octanoic and decanoic acids which have antimicrobial agents that will limit the growth of the yeast itself and other microbes. The acids produced by the yeast because they have antimicrobial properties can be used as a food preservative. Yeast cell shapes vary, namely round, oval, cylindrical or rod, curved triangle, bottle-shaped, apiculate or lemon shape, forming a pseudomycelium. Yeast can grow in concentrated solutions, for example in excess sugar, salt and acid solutions. Yeast has antimicrobial properties so it can inhibit the growth of bacteria and mold. The existence of properties that are resistant to environmental stress (sugar, salt, and excess acid) makes yeast able to survive or compete with other microorganisms.

Macroscopic morphological observations of yeast were carried out by observing colony morphology which included shape, texture, color, surface, elevation and edges. While microscopic morphological observations were carried out by observing yeast cells which included cell shape, size, budding, presence or absence of hyphae or pseudohyphae, and the type of spores obtained.

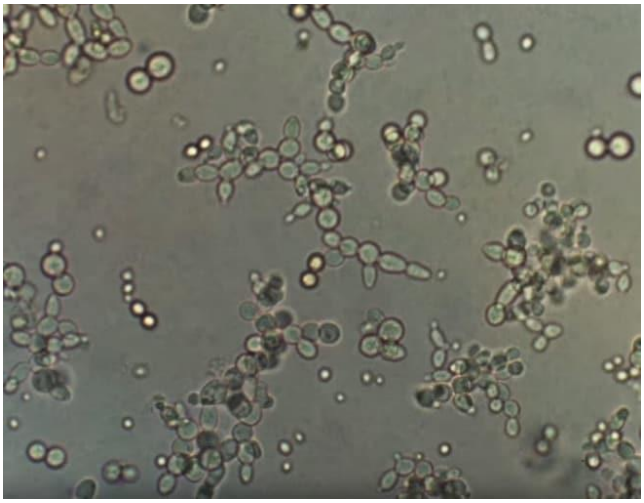


Figure 3.1 Appearance of yeast under the microscope

Figure 3.2 Appearance of yeast colony morphology

Objective :

1 Observing Yeast, Rhizopus sp., Aspergillus sp., and Penicillium sp. preparations and their morphology.

Experiment Methodology:

A. Tools :
Microscope
Preparation page
Cover glass

B. Bahan :
Yeast preparation, Rhizopus sp. preparation, Aspergilus sp. preparation, dan Penicillium sp. preparation.

C. Ways of Work
1. Cleaning glasses with tissues
2. Drops of glass preparations with aquadest in the middle
3. Taking a small amount of mold culture with a preparation needle
4. Laying on a glass preparation that has been dripped with aquadest
5. Separation with 2 preparation needles if the mycelia collects
6. Observation weak magnification first
7. Observation with moderate magnification
8. Observation of the desired parts with a strong magnification
9. Depiction of parts and provision of information.

D. Discussion :
A. Definition of Aspergillus sp
Characteristics of Aspergillus sp
Explanation of the observed Aspergillus sp morphology (in the picture not in the photo *give a description of what appears in the picture seen from the microscope) and Aspergillus sp morphology standards *from the literature
Advantage and Disadvantages Aspergilus sp

B. Definition of Penicillium sp..

Characteristics of Penicillium sp..
Explanation of the morphology of Penicillium sp.. from the observations (in the picture not in the photo *give a description of what appears in the picture as seen from the microscope) and the standard morphology of Penicillium sp.. *from the literature
Advantage and Disadvantages Penicillium sp..

C. Yeast definition
Characteristics of Yeasts
Explanation of observed Yeast morphology (in the picture not in the photo *give a description of what appears in the picture seen from the microscope) and standard yeast morphology *from the literature
Advantages and Disadvantages of Yeast (both in food and non-food)Kesimpulan

Conclusion

E. Bibliography

NOTES :

Group Turn Distribution: (K3 Laboratory 3rd floor)

Turn	Group	Time
1-3a	1,2	09.00 – 09.30
2 -3a	3,4	09.30 – 10.00
3 -3a	5,6	10.00 – 10.30
1-3b	1,2	13.00 – 13.30
2 -3b	3,4	13.30 – 13.30
3 -3b	5,6	13.30 – 15.00

EVENT 4 – Detection of Bacteria by Gram Stain

Literature Review

Gram staining is one of the most important staining techniques in microbiology. Gram positive bacteria will show a violet color when stained, while gram negative bacteria will produce a red color. Gram positive bacteria have a higher peptidoglycan content, while gram negative bacteria have a higher lipid content.
The basic principle of gram staining is the ability of the bacterial cell wall to bind crystal violet dye during solvent administration. The first step in gram staining is the use of crystal violet dye to pre-stain the slide. The next step, also known as fixation, involves using iodine to form a crystal violet-iodine complex to prevent easy loss of dye. The next step is decolorizing, often ethanol and acetone solvents are used to wash off the dye. The color washing period must be considered, because prolonged exposure to solvents will remove the color on the bacterial wall. In the initial stage, the bacteria will bind to the crystal violet dye, but when washed with a solvent, the cell wall of gram-negative bacteria which is composed of lipids will decay. With the decay of the bacterial lipid layer, the gram-negative bacteria will lose their primary dye. Conversely, the cell wall of gram-positive bacteria will become dehydrated due to the presence of solvents, as a result the cell wall pores will close and prevent the diffusion of the violet-iodine complex, so the bacteria will remain stained.

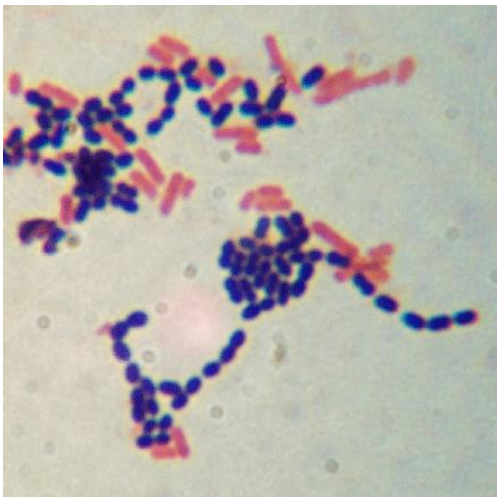


Figure 4.1. Bacterial gram stain results. Violet color indicates gram positive bacteria, red color indicates gram negative bacteria.

General Purpose:

1 Determining Gram Positive or Negative *Escherichia coli* and *Staphylococcus Aureus* bacteria

Particular Purpose :

1. Able to properly prepare tools/materials
2. Able to make smear preparations properly
3. Able to perform Gram stain technique with accuracy
4. Able make observations using a microscope
5. Able to determine the results observed in the Gram staining technique

Experiment Methodology :

A. Alat :
   1. Microscope
   2. Spirits Burner
   3. Preparatory Glass

4. Drop pipette
5. Stopwatch
6. Hairdryer
7. Test tube
8. Match
9. Tissue
10. Marker
11. Bunsen
12. Ose

B. Ingredients :
   1. Alcohol
   2. Aquadest
   3. Kultur : Escherichia coli dan Staphylococcus Aureus
   4. Cat A : Violet Chrystal
   5. Cat B : Lugol
   6. Cat C : Alcohol 95%
   7. Cat D : Safranin
   8. Tweezers/forceps
   9. Spirtus lamp

C. Ways of Work
   1. Cleaning glasses with tissues
   2. Prepare pure cultures of bacteria to be stained.
   3. Aseptically take one review of the needle loop for bacterial culture on the surface of the glass preparation.
   4. Flatten it using an ose needle.
   5. Heat fixation by passing the glass slide over the Bunsen flame 3 times. Fixation is used to kill bacteria but still maintain the shape and components of the cell.
   6. Drop two drops of primary dye A (Crystal Violet) on the bacterial review, and wait for 1 minute.
   7. Rinse the primary dye using distilled water and wait until it is dry enough (hairdryer).
   8. Drop B-mordant solution (Lugols Iodine) on the surface of the bacterial review, and wait for 1 minute.
   9. Rinse the mordant solution using distilled water and wait until it is dry enough (hairdryer).
   10. Drop the decolorizer C (ethanol) solution evenly on the bacterial review, wait for 10 seconds.
   11. Rinse the decolorizer solution using distilled water and wait until it is dry enough (hairdryer).
   12. Apply the secondary dye (Safranin) evenly to the bacterial smear, and wait 20 seconds.
   13. Rinse the secondary dye using distilled water and wait until it is dry enough.
   14. After drying, cover the surface of the review with a glass cover and the sample is ready to be observed under a microscope.

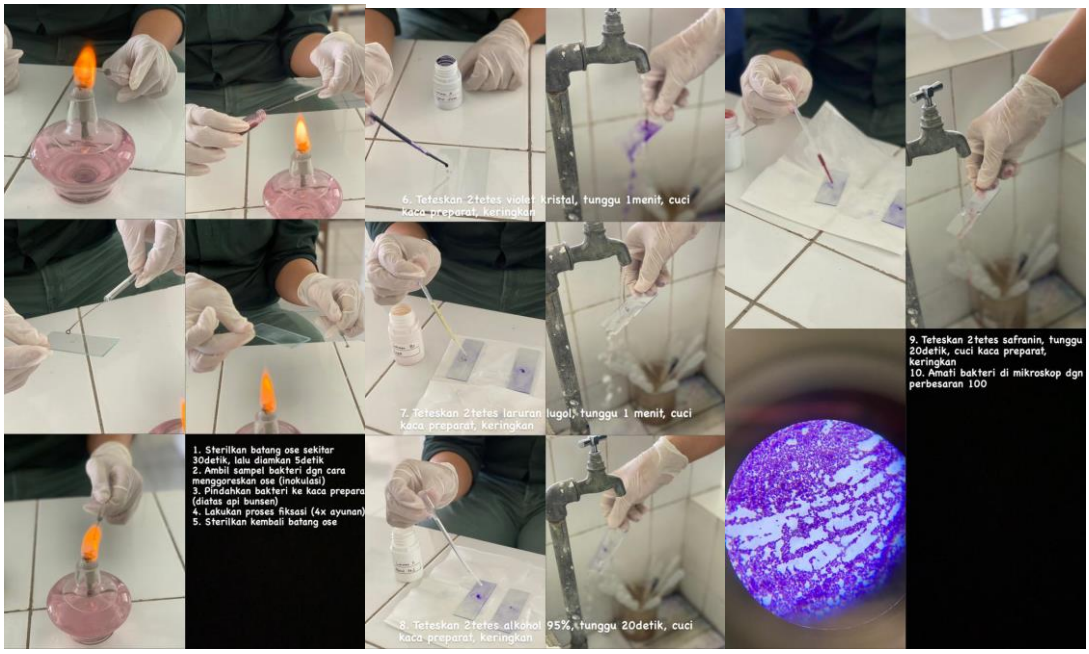


Figure 4.2. Gram Stain Groove

D. Gram staining picture (picture : not photo)
E. Discussion
   Gram stain definition
   Types and types of several bacterial staining techniques
   The solution used in the gram staining lab
   - Paint A: composition, function and use
   - Paint B : etc…

   Practicum Result
   - Bacteria I: the color change that occurs When observed, the cause of the color change, including gram positive/negative bacteria
     Image: not a photo
     Give a description: the name of the bacteria, shape, magnification, color of the colony
   - Bacteria II: color change that occurs When observed, the cause of the color change, including gram positive / negative bacteria
     Image: not a photo
     Give a description: the name of the bacteria, shape, magnification, color of the colony

F. Conclusion
G. Bibliography
H. Appendix
   Photo of bacteria from reference

EVENT 5 – Observation of Influential Factors in the Growth of Fungi and Bacteria

Literature Review

Each type of microorganism has a different growth time, but there are 4 main phases, namely the lag phase, the exponential phase, the stationary phase, and the death phase. The lag phase is the phase in which microorganisms adapt to the surrounding environment. In this phase, microorganisms will try to synthesize RNA, enzymes, and other metabolites that support their growth environment. This phase has a different time between each microorganism, depending on several factors, including:

a. adaptive ability of each microorganism (whether there are many damaged microorganism cells, so it takes longer for cell repair)
b. the difference in conditions between the new place/media and the old one
c. meet the needs of microorganisms for growth

Exponential phase / log phase will begin when the needs of microorganisms to grow have been met so that cell division occurs. This phase can be detected when the number of microorganism cells increases at least 2 times the original number. In this phase, microorganisms are at their peak so that beneficial microorganisms can be used in food production, research, etc. Meanwhile, pathogenic microorganisms will damage food and cause foodborne illness or food allergy.

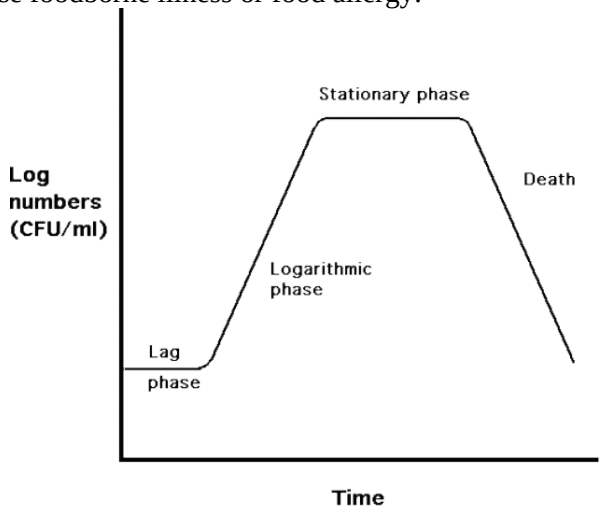


Figure 5.1 Microorganism growth curve

The growth of microorganisms is influenced by several factors, namely:

a. Intrinsic factor
   Conditions that naturally exist in food ingredients that affect the growth of microbiota, including:
   1. pH
   2. Aktivitas Air [Water activity (aw)]
   3. Redox potential
   4. Nutrient content
   5. Antimicrobial content
   6. Biological structure

b. Extrinsic factor
   External conditions in which food is stored, such as:
   1. Storage temperature
   2. Relative humidity [Relative humidity (RH)] in storage

3. Concentration and presence of gas in storage

c. Implicit factor
Factors related to the inherent characteristics of microorganisms, including how microorganisms interact with other microorganisms, as well as their interactions with food ingredients. There are two characteristics of the interaction of microorganisms, namely:
1. **Antagonism**: when there is competition between microorganisms in foodstuffs in fulfilling nutrients, production of inhibitors etc
2. **Synergism**: when the growth of a microorganism supports the growth of other microorganisms a suitable environment is formed for the growth of other microorganisms

d. Processing factor
Processing can change the composition and condition of the intrinsic factor and reduce the number of microorganisms, for example:
1. high temperature
2. Low temperature
3. Drying
4. Fumigation, etc

General Purpose:
1. Observing microbial growth in food with various treatment conditions
2 Observing the morphology of growing microbiology.

Experiment Methodology:

A. Tools :
Food ingredients sample* 2 pieces

B. Methods

Group	Ingredients	Temperature	Packaging
1	Butter Cake (Made in market) Butter Cake (Brand)	Room Temperature	Open
2	Butter Cake (Made in market) Butter Cake (Brand)	Room Temperature	Closed/vacuum
3	Butter Cake (Made in market) Butter Cake (Brand)	Chiller	Open
4	Butter Cake (Made in market) Butter Cake (Brand)	Chiller	Closed/vacuum
5	Bread (Cheap) Bread (brand)	Room Temperature	Open
6	Bread (Cheap) Bread (brand)	Room Temperature	Closed/vacuum
7	Bread (Cheap) Bread (brand)	Chiller	Open
8	Bread (Cheap) Bread (brand)	Chiller	Closed/vacuum
9	Donut (made in market) Donut (brand) *can be replaced with the same type	Room Temperature	Open
10	Donut (made in market) Donut (brand) *can be replaced with the same type	Room Temperature	Closed/vacuum

11	Donut (made in market) Donut (brand) *can be replaced with the same type	Chiller	Open
12	Donut (made in market) Donut (brand) *can be replaced with the same type	Chiller	Closed/vacuum

Observational Notes

Days	1	2	3	4	5	6	7	8
Ingredients : *ex : butter cake								
Documentation								
Description/Changes								
Ingredients : *ex : butter cake								
Documentation								
Description/Changes								
---- etc								

C. Discussion

- Factors that affect the acceleration of the growth of bacteria and fungi
- Factors that inhibit the growth of bacteria and fungi
- The fastest treatment makes the fungus/bacteria grow
- The slowest treatment makes mold/bacteria grow
-

D. Conclusion
E. Bibliography
F. Appendix
Photo of bacteria from reference

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