MICROBIAL PHYSIOLOGY LAB

MICR 3146

(CRN's: 11348,11349, 11350, 14685)

Fall 2024

COVID-19 PRECAUTION STATEMENT

- Please stay home if you have been diagnosed with COVID-19 or are experiencing COVID-19 symptoms.
- If you are feeling unwell, please let me know as soon as possible, so that we can work on appropriate accommodations.
- If you have tested positive for COVID-19, you are encouraged to report your results to covidaction@utep.edu, so that the Dean of Students Office can provide you with support and help with communication with your professors. The Student Health Center is equipped to provide COVID-19 testing.
- The Center for Disease Control and Prevention recommends that people in areas of substantial or high COVID-19 transmission wear face masks when indoors in groups of people.
- The best way that Miners can take care of Miners is to get the vaccine. If you still need the vaccine, it is widely available in the El Paso area, and will be available at no charge on campus during the first week of classes.
- For more information about the current rates, testing, and vaccinations, please visit epstrong.org.

- https://arstechnica.com/science/2021/06/even-mild-covid-in-young-people-often-leads-to-long-term-symptoms-study-finds/

- "concern about finding mild cases in young people leading to long-term problems. “It is worrying that non-hospitalized, young people (16–30 years old) suffer potentially severe symptoms, such as concentration and memory problems, dyspnea [difficulty breathing] and fatigue, half a year after infection,” the authors wrote. “Particularly for students, such symptoms might interfere with their learning and study progress…"

- If you are not familiar with long-haulers syndrome described in the above study, this 60 Minutes story illustrates it in great detail:

- https://www.youtube.com/watch?v=0gLmMPOHDwM

- https://www.buzzfeednews.com/article/amberjamieson/florida-covid-delta-variant-surge

- While with the original coronavirus, an infected person was expected to infect two to three others, a person infected with Delta is expected to infect anywhere from five to nine.
- https://www.nytimes.com/2021/08/17/health/covid-vaccinated-infections.html?referringSource=articleShare

TABLE OF CONTENTS

Microbial Physiology Lab
Fall 2023

INSTRUCTOR:	Dr. Sangeeta Tiwari, Department of Biological Sciences
OFFICE:	Bioscience Research Building, Room 3.148
PHONE:	Office: 747-8776
EMAIL:	stiwari@utep.edu
OFFICE HOURS:	By appointment, stiwari@utep.edu
Lab Instructors: (TA)	Ana Vargas (Monday Section, CRN: 11348) amvargas2@miners.utep.edu
	Cassandra Almanza (Tuesday Section, CRN: 11349) calmanza@miners.utep.edu
	Roxana Garcia (Thursday Section, CRN:11350, 14685) rlgarciamejia@miners.utep.edu

LABORATORY: Biology Building, Room B411

LAB TEXT: Lab manual posted on Blackboard

LAB OBJECTIVES

A mixture of bacterial colony morphologies grew on a petri dish. The objective of this lab is for each group of students to utilize microbiological techniques in order to identify selected bacterial colonies. You will use common and advanced lab techniques to examine the physiology of the cell and the many processes the cell uses to produce energy and the metabolites required for survival. You will examine a multitude of techniques, including the principles behind, the uses for, and interpretation of these and other techniques. Understanding this information will be essential to your performance in this class.

LAB GOALS

1. Learn the fundamental concepts in microbial physiology
2. Apply the concepts you've learned
3. Extrapolate information and facts from what you already know
4. Communicate your understanding of microbiology
5. Learn to think critically

LABORATORY EXERCISES

The laboratory exercises are designed to teach fundamental microbiology techniques, including sterile technique, bacterial isolation and culture, macromolecule purification, and identification of microorganisms. The laboratory will meet in Room B412 of the Biology Building, starting the second week of class. The first session will include a

REQUIRED laboratory safety lecture. NO ONE will be allowed to work without this training.

LABORATORY POLICIES

SAFETY. Rules exist for your safety, since the organisms we will be using in the lab can make you sick. The first laboratory meeting will include a REQUIRED safety lecture. You will not be permitted to work in the lab until this training has been completed. For Lab PPE, you are **required** to **provide your own lab coat**, which you must bring to the lab EACH WEEK, gloves will be provided. Only closed-toed shoes will be permitted and long hair must be pulled back OR placed in a hairnet. If you do not meet the dress requirements you will be asked to leave. No food, drink, or application of cosmetics will be permitted in the laboratory.

MISSING LAB. You will only receive credit for a lab report **if you attended the lab itself**. Do NOT miss lab, attendance will be taken each week and a quiz given at the start of class. Do NOT be late for class; excessive tardiness may result in not being given the quiz at the TAs discretion. Since there is only one lab section you cannot make up the lab nor the quiz after it has been given. If you miss a lab for a reasonable excuse, notify your TA. Going to the doctor is NOT a reasonable excuse!! Schedule your appointment for another time. Lab meets at the same time every week and cannot be made-up. **DO NOT** schedule **anything** during this time.

LATE LAB REPORTS. Each lab report is due ONE WEEK after the laboratory exercise has been concluded. It must be turned in to your TA at the beginning of lab. Lab reports will NOT be accepted on any other day. Failure to turn in a lab report will result in an automatic zero.

COURTESY. As a courtesy to your classmates, please give your full attention to all speakers and limit your in-class discussions to topics related to microbiology. Cell-phones and pagers must be turned off during class sessions and labs. The use of laptop or notebook computers or tablets during class and lab sessions is limited to note-taking and coursework only – refrain from browsing the internet or checking your email during class. Please be on time for class – roll may be taken at the start of each class session.

ACADEMIC DISHONESTY. It is the official policy of the University of Texas at El Paso that academic dishonesty is a completely unacceptable mode of conduct and will not be tolerated in any form. Scholastic dishonesty includes, but is not limited to cheating, plagiarism, collusion, the submission for credit of any work or materials that are attributable in whole or in part to another person, taking an examination for another person, any act designed to give unfair advantage to a student or the attempt to commit such acts. All persons involved in academic dishonesty will be disciplined in accordance with University regulations and procedures. Please see http://www.studentaffairs.utep.edu for details

DISABILITIES AND MEDICAL CONDITIONS. If you have or suspect you have a disability and need an accommodation, please contact the Center for Accommodations and Support Services (CASS) at 747-5148, at cass@utep.edu or go to Union Building East, Room 106. **If you have medical condition and cannot attend lab in person you need approval from CASS.**

GRADING SYSTEM
Your grade will be based on 7 lab reports, 9 quizzes, midterm and final exams. It is recommended that you maintain a notebook. However, this is for your own study purposes and will not constitute a grade in the class. Lab reports are required and will be due the following week after the lab.

1. QUIZZES. You must read each day's lab manual assignment BEFORE COMING TO LAB. The quizzes are designed to test your knowledge of the material presented in lab and in your reading assignments. Your TA will give the quiz at the beginning of each laboratory period, so please be on time to lab or you will miss that day's quiz. A total of 10 quizzes will be given. One quiz will be dropped and the best 9 counted towards your grade.

2. LAB REPORTS. Lab Reports must be turned in ONE WEEK after each lab session. EACH GROUP will be required to turn in ONE common Lab Report.

3. EXAMS. Two exams will be given during the laboratory time. The exams are worth a total of 100 points. The exam will test your understanding of all of the materials covered in the laboratory and your ability to APPLY the concepts you have learned.

In summary, the grading policy is as follows:

3 Lab Reports (20 pts each)	60 Points
9 Quizzes (5 pts each)	45 Points
Midterm	22.5 Points
Final	22.5 Points
Total	**150 Points**
*Extra Credit (Lab notebook)	10 Points

Grade	**Points**
A	120-150
B	90-119
C	60-118
D	30-59
F	29-below

Materials for this lab:

- Lab Coat (**Required**)

    - If you do not have a lab coat, you will not be allowed in the laboratory and there are no make-ups for the labs.
- Sharpie (**Required**)
- Notebook
- Lab Manual
    - The lab manual is available on Blackboard. All protocols will be discussed in the lab prior to the lab in which the protocol is performed. It is recommended that you perform all protocols on paper, prior to lab and before attempting the protocol in lab. This will give you an idea of where and what kinds of problems to expect in lab. This is also a good way to learn the protocols and to understand where the theory of these protocols applies.

BASIC LABORATORY SAFETY

The laboratories planned for this semester are safe. However, we will be working with physical and chemical hazards that require safe handling conditions. Please take a few minutes to review these practices. In the future, as you progress in your scientific training, you will be faced with handling HAZARDOUS chemicals and substances. Therefore, it is essential that you develop good laboratory practices now. In general, if you are not sure of something, ask your instructor! It may prevent a problem.

Carelessness and ignorance are the most common causes of personal injury in the laboratory. It is essential that a student follow the instructions given by instructor.

GOOD LABORATORY PRACTICES RELATED TO YOUR PERSONAL SAFETY:

1. Familiarize yourself with the health and safety hazards of the equipment and chemicals with which you are working. Chemical manufacturers provide a Safety Data Sheet (SDS) for each chemical; these are available for your review. The SDS describes potential hazards associated with working with a substance and gives the emergency response procedures. The SDS should be reviewed prior to the laboratory.

2. Handle hazardous chemicals carefully. Do not move them around the room uncovered. Place them towards the back of the lab bench so there is less chance that they will be knocked over. Never return unused chemicals to the stock bottle. Do not use unlabeled chemicals.

3. Do not taste or inhale any material. Work with chemicals in the fume hood if appropriate (your instructor will note when this is the case).

4. You should wear appropriate clothing to the lab. You MUST wear closed-toed shoes – no sandals, flip-flops, or similar footwear is allowed. If a chemical is spilled or something is dropped on the floor, your foot will be protected. Personal Protective Equipment (PPE) must be worn where appropriate, as indicated by your instructor.

5. If you have long hair, tie it back so that there is no chance it will interfere with your work or get contaminated by a chemical and/or catch on fire.

6. Familiarize yourself with the eyewash station and its use, as well as the locations of fire extinguishers and other emergency equipment and of the exits.

7. Never pipet by mouth!

8. Do not eat or drink in the lab.

9. Remove any PPE before leaving the lab.

10. Wash your hands before leaving the lab. It is very easy to carry small amounts of potentially hazardous chemicals or biological materials on your hands. If you then proceed to eat lunch or dinner without washing your hands, you could ingest these substances unknowingly.

11. Keep work areas clean. Do not pour any chemical down the sink drains. Dispose of chemicals only in labeled containers designated for their disposal.

EMERGENCY RESPONSE PROCEDURES

1. If you are injured, notify the instructor IMMEDIATELY.

2. If a chemical or biological spill occurs, notify the instructor IMMEDIATELY.

3. If the fire alarm is activated, IMMEDIATELY leave the building with your instructor and await further instructions.

4. Emergency contact numbers:

University Police	ext. 5611	3118 Sun Bowl Drive
Main Office, Biological Sciences Department	ext. 5844	Bioscience Research Bldg., Rm. 2.120
Environmental Health and Safety	ext. 7124	Carl Hertzog Building, Rm. 170
Facilities Services Emergency	ext. 7187	3121 Sun Bowl Drive
Life Threatening Situations	911	

Revised 01/28/2013

BIOSAFETY PRACTICES

Laboratory Biosafety Levels

The CDC and NIH describe four biosafety levels (BSL) for activities involving infectious agents. The levels are designated in ascending order by degree of protection provided to lab personnel, the environment, and the community. BSL1 is for work with infectious agents that pose minimal or no hazards, while BSL4 is for work with the infectious agents that pose the greatest hazard. Each level recommends facility design, lab practices, and safety equipment appropriate for working with the infectious agent involved. BSL1 through BSL4 are discussed briefly below. A more exhaustive discussion of biosafety level criteria can be found in CDC/NIHs "Biosafety in Microbiological and Biomedical Laboratories." The pathogens studied in the Pathogenic Microbiology (MICR 3144) wet labs are Biosafety Level 1 and Biosafety Level 2 organisms. However, you will use BSL-2 practices and procedures.

Biosafety Level 1 (BSL-1)
BSL-1 practices, safety equipment, and facilities are appropriate for undergraduate teaching laboratories using microorganisms not known to cause disease in healthy adult humans. BSL-1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended.

Biosafety Level 2 (BSL-2)
BSL-2 practices, safety equipment, and facilities are recommended for clinical, diagnostic, research, or teaching laboratories involving moderate risk agent associated with human disease of varying severity. The primary hazards to lab personnel working with these agents include accidental skin or mucous membrane exposures, or ingestion of infectious materials. BSL-2 is appropriate when work is done with any human-derived blood, body fluids, or tissues where the presence of an infectious agent may be unknown. Primary barriers recommended include biological safety cabinets (BSC) and personal protective equipment (PPE). Secondary barriers recommended include waste decontamination facilities.

Biosafety Level 3 (BSL-3)
BSL-3 practices, safety equipment, and facilities are recommended for clinical, diagnostic, research, or teaching laboratories involving indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. Primary hazards to lab personnel working with these agents include autoinoculation, ingestion, and exposure to infectious aerosols. Primary barriers that must be used include BSCs or other enclosed equipment. Secondary barriers for this level include controlled access to the laboratory, a specialized ventilation system, and waste decontamination facilities.

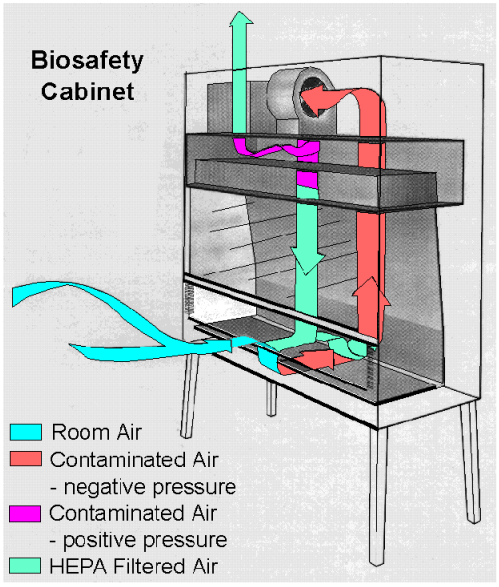
Biosafety Level 4 (BSL-4)

BSL-4 represents maximum containment and is required for dangerous and exotic agents that pose a high risk of life-threatening disease. These are agents that may be transmitted via the aerosol route and for which there is no available vaccine or therapy.

Biological Safety Cabinets (BSC's)

Biological safety cabinets (BSC's) are among the most effective, as well as the most commonly used, primary containment devices in laboratories working with infectious agents. The BSC is designed to capture and contain any infectious particulates or aerosols generated within the BSCs interior and exhaust them through a high-efficiency particulate air (HEPA) filter either into the laboratory, or to the outside.

The three general types of BSC's are available (Class I, II, and III). More detailed information on BSCs can be found in CDC/NIHs "Biosafety in Microbiological and Biomedical Laboratories." The BSC's available to us in Room B412 of the Biology Building are Class II, Type A2. The Class II BSC has an increased face velocity relative to the Class I BSC and the additional advantage of providing protection to the research material by HEPA filtration of the airflow into the cabinet across the work surface. This type of cabinet will protect the user, environment, and the research material and is suitable for work with moderate- to high-risk agents (in the case of our laboratory, Biosafety Level 2). Class II BSCs are classified into two types: A and B. Type A cabinets like ours exhaust the cabinet air into the laboratory. Since the air is re-circulated within the laboratory, volatile or toxic chemicals and radionuclides should not be used inside this type of cabinet. A diagram of airflow circulating in a BSC is pictured below.



Proper use of biological safety cabinets

As with any other piece of laboratory equipment, personnel must be trained in the proper use of the BSC. Of particular note are those activities that may disrupt the inward directional airflow through the work opening of the Class II cabinets. Repeated insertion and withdrawal of the workers' arms into and out of the work chamber, opening and closing doors to the laboratory, improper placement or operation of materials or equipment within the work chamber, or brisk walking past the BSC while it is in use are demonstrated causes of the escape of aerosolized particles from within the cabinet. Strict adherence to recommended practices for the use of the BSC is important in attaining the maximum containment capability of the equipment and maintaining mechanical performance of the equipment itself.

The following steps are essential for proper use of pathogenic organisms in the BSC:

1. Operate the blower in the BSC for five minutes before you begin work in the cabinet to purge any air-borne contaminants.

2. Disinfect the work surface of the BSC before use. A 70% ethanol or a freshly prepared 10% chlorine bleach (sodium hypochlorite) solution is a suitable disinfectant. Keep in mind that the bleach solution is corrosive and can damage the stainless steel work surface. Therefore, it must be followed by a thorough wash with water to prevent corrosion.

3. Set up your work surface. You will need a small biohazard bag for solid waste and a beaker or other vessel (containing your disinfectant) for liquid waste. Keep a squirt bottle of your disinfectant handy in the event that a spill might occur. Do not use a Bunsen burner or other source of flame in the BSC. Instead, you will find a ceramic hot plate in the BSC for use in drying your samples onto the slides prior to Gram staining. Place any slides, tubes, or plates needed for the day’s experiments in the BSC prior to beginning work.

4. Be careful not to place any objects on the air intake or exhaust grills, as this will disrupt the airflow. Similarly, always conduct your work at least four inches beyond the opening of the BSC.

5. Always wear a lab coat, eye protection, gloves, and other PPE as indicated by your instructor.

6. In the BSC, work slowly and deliberately. Minimize the movement of your hands into and out of the BSC to avoid disturbing the airflow.

7. Spills happen, but with much less frequency if you are working carefully. If you spill ANYTHING, either inside or outside of the BSC, report it to your instructor IMMEDIATELY. He or she will talk you through the proper cleanup for your spill. You will also be taught some basic cleanup skills during the laboratory safety training, but always enlist the help of your TA. If you’re unsure of what to do or

have any questions, please call Dr. Tiwari at extension 8776 or Environmental Health and Safety (EH&S) at extension 7124.

8. Always properly dispose of tips, tubes, wipers, and any other materials used in the BSC, especially those that have come in contact with biological agents.

9. After completing your work inside the BSC, operate the blower for five minutes before to purge any air-borne contaminants.

10. Remove your PPE and thoroughly wash your hands and arms before leaving the lab.

Revised 01/28/2021.

I hereby acknowledge receipt of notice of the availability of the Microbial Physiology Laboratory safety manual. I agree to access and read the manual and abide by all policies, safety rules, and procedures defined or referenced in the document.

_____ _____

Name and ID number *Date*

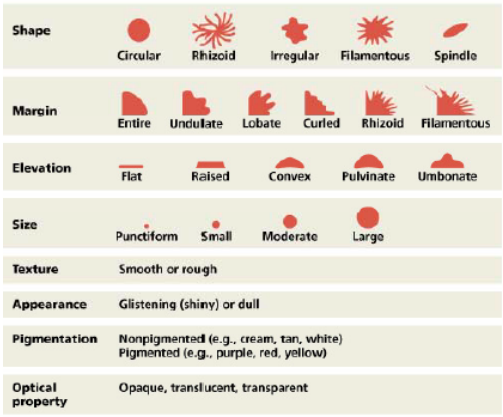
Microbial Physiology Lab
Fall 2024

Bacterial Isolation and Antibiotic Susceptibility
Lab 1 - Week of September 9, 2024

Introduction:

The physiology of microbes is routinely used in research, reference and clinical labs to identify a bacterium. The way that a microbe responds to various food sources and environmental stressors can tell you a lot about the physiology of the bacterium. Since the genome of each bacterial species encodes for a unique set of proteins that govern the existence of the bacterium, amassing a collection of data on the pathways and molecules contained by the bacterium can narrow or even specially identify a bacterial agent. Throughout the course of this semester, you will utilize multiple analytic techniques to amass a critical amount of information to identify a given bacterium.

In this lab, you will select the bacterium that you wish to study for the rest of the lab exercises and begin tests to identify the bacterium. Due to the physiology of bacteria, their colony shape and color vary when grown on various solid media. These properties can be distinctive for different bacteria or shared among different species. In addition, alterations/mutations in the bacteria can be reflected in alterations in the colony morphology. It is always important to not each of the following whenever handling an unknown bacterium.



Antibiotics function by targeting a specific step in the metabolic or physiology pathways of bacteria. There are numerous antibiotic classes each with different efficacies on different classes of bacteria based on the pathways the bacteria utilizes. Therefore, antibiotic susceptibility testing can give some basic information about the physiology of unknown bacteria. This technique is not only routinely performed in clinical labs to determine which antibiotic to give a patient but also in reference and research labs to identify unknown bacteria. For example, extreme susceptibility to β-lactam antibiotics would suggest that the bacterium is gram-positive, due to the heavy reliance on the peptidoglycan layer in the bacterial cell wall and no outer membrane to prevent absorption.

The purpose of this lab is two-fold; 1) to select an unknown bacterium, and 2) to make assumptions about its identification based upon its physiology as revealed by colony morphology and antibiotic susceptibility.

Materials:

Blood agar plate containing unknown bacterial isolates (E coli, Staph aureus, Staph epidermidis, or Staph saprophyticus) labeled A-D in four separate sections
Inoculating loop/needle
Broth
Mueller-Hinton Agar plates
Culture tubes
20% sterile glycerol
Eppendorf tube
Antibiotic containing discs
Ruler

Protocol:
Colony selection (**Week of September 9, 2024**)
*Highlighted methods will be completed by TA.

Working in the hoods to keep everything sterile, aliquot 2 mL of medium into sterile tubes and cap with sterile caps.

Note: It is important to work in the hood and keep the medium sterile so that only the inoculated bacteria will grow.

Use the inoculating loop to select a colony and transfer it, and ONLY it, into the 2 mLs of broth in a culture tube. Place at 37°C.

Grow for 24 hours at 37°C.

Cultures will have settled, so resuspend the culture and transfer 500 uL to a sterile Eppendorf tube and add 500 uL 20% sterile glycerol, mix well and place in the freezer.

Antibiotic susceptibility (**Week of September 9, 2024**)

Inoculate 500 uL of broth with a scraping from your frozen stock.

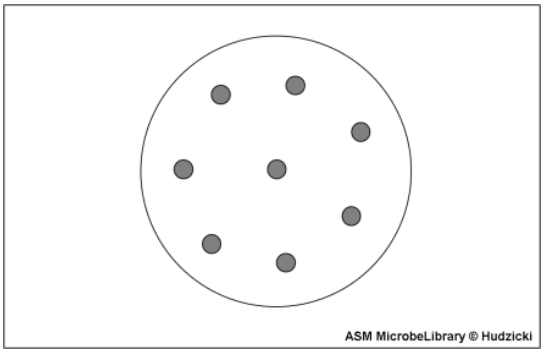
Grow for 2 hours at 37°C in the shaking incubator.

After 2 hours growth, remove 200 uL of culture and spread on an agar plate.

Allow the plate to dry for 5-15 minutes with the lid on.

Carefully place the antibiotic containing discs on the surface of the plate. DO NOT TOUCH THE DISPENSER TO THE PLATE OR IT WILL BECOME CONTAMINATED.

Place plates at 37°C.



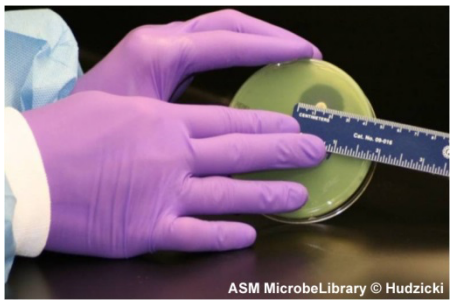
Incubate plates for 1-2 days at 37°C. TA will remove plates from 37°C and place at 4°C.

Students will record results (as indicated below) during the week of September 18, 2023.

Measuring zone sizes

1. Following incubation, measure the zone sizes to the nearest millimeter using a ruler or caliper; include the diameter of the disk in the measurement

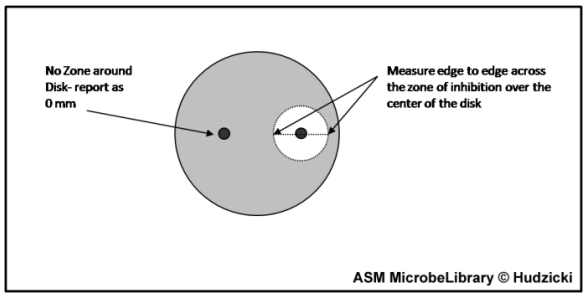
2. When measuring zone diameters, always round up to the next millimeter.



3. All measurements are made with the unaided eye while viewing the back of the petri dish.

4. If the placement of the disk or the size of the zone does not allow you to read the diameter of the zone, measure from the center of the disk to a point on the circumference of the zone where a distinct edge is present (the radius) and multiply the measurement by 2 to determine the diameter.

5. Growth up to the edge of the disk should be reported as a zone of 0 mm.



Lab Report: (at a minimum address the following)

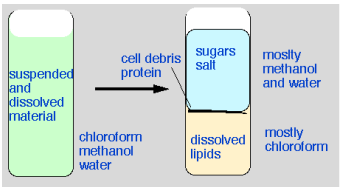
Why are you doing this lab?
How did you do this lab?
What were the results of this lab?
What do the results mean?
Where there any problems during the lab/with the results?
How can these results help to discover the identity of your unknown bacterium?
Tabulate colony characteristics and growth inhibition.

Microbial Physiology Lab
Fall 2024

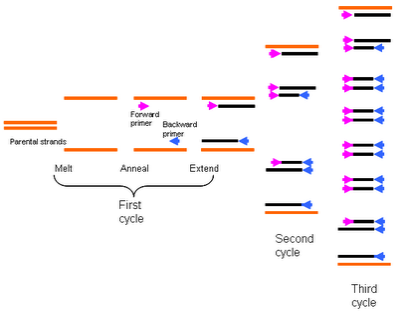
DNA Isolation, PCR amplification and gel electrophoresis
Lab 2, 3, 4 – starting Week of September 16, 2023

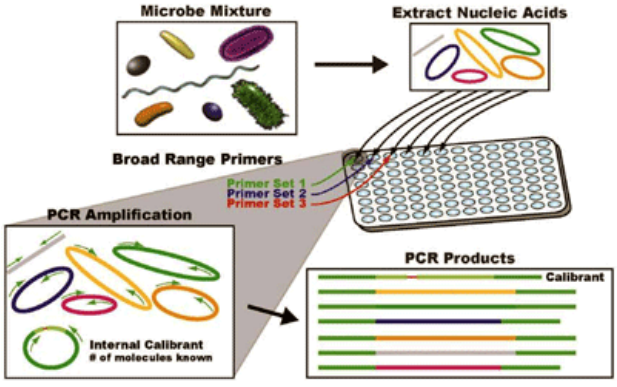
Introduction

DNA isolation has become sensationalized on television "cop" shows in their ability to isolate DNA from any sample, no matter how minute. The old method (which you will use) of isolating DNA required a large number of organisms in order to end up with sufficient sample for further analysis. However, DNA isolation is now so common that commercially available kits make short work of purifying DNA from any organism by binding DNA to a spin-column matrix. However, it is important to understand the process behind the kit as not all organism DNA is readily purified using the kit. Indeed, this is one scientific principle the TV shows got right. Highly hydrophilic proteins, nucleic acids, sugars and salts will be dissolved in the phenol fraction but excluded from the chloroform. Following centrifugation, the two immiscible solvents will separate to form a discontinuous mixture with the aqueous portion on top and the heavier chloroform on the bottom. One can then separately obtain organics verses sugars/proteins. Addition of ethanol then causes nucleic acids to precipitate out of solution.

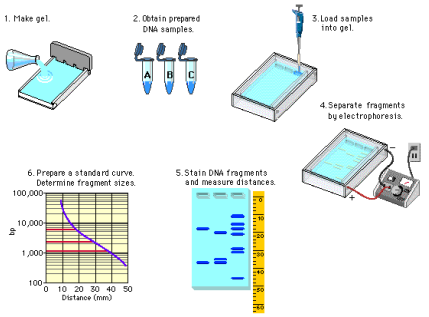


The polymerase chain reaction (PCR) is a cyclical method of amplifying DNA using the same replication machinery as the cell. Remember that the DNA polymerase must have a primer to start its enzymatic activity. This primer varies for different cells but always indicates the starting point for the DNA polymerase. In PCR, we can define that starting point by providing the primer designed to binding to a specific site on the DNA template. If we provide two primers that bind to opposite strands of the DNA then after several replication cycles the largest majority of the PCR products terminate after the primers and we are only left with significant quantities of what lies between the two primers. Therefore, using a pair of primers that is specific for a given DNA sequence, we can amplify what "lies between" the primers. Given that what "lies between" the primers is governed by the genetic sequence of the organism and that each organism has a different genetic sequence, the distance between the primers, and hence the length of the PCR product, should be different for each bacterium. Therefore, using PCR, we can amplify the DNA from a mixed culture of bacteria to see how many different genomic sequences are present or even purify the different lengths of PCR products representing the different organisms. If you are not familiar with PCR yet, look up the procedure of denaturing, annealing, and extension.





In order to visualize these fragments, gel electrophoresis separates nucleic acids (or proteins) by size by applying an electric current to a gel. The gel is a solid yet porous matrix in which the molecules are contained and separated by the action of the electric current. For DNA electrophoresis, acrylamide or agarose are generally used to make the gels. For acrylamide gels, it is necessary to use a cross-linker to make the gel. Acrylamide is a neurotoxin and should be handled carefully. Agarose is a very complex carbohydrate and will solidify after heating so the use of cross-linkers is not necessary. In order to visualize the DNA contained in the gels, the most common choice is ethidium bromide (EtBr).



Important Safety Note: EtBr is an intercalating agent and a known mutagen and carcinogen.

When exposed to UV light, EtBr will give an orange color, making it possible to visualize the DNA. Therefore, the purpose of this lab is two-fold; 1) to familiarize you with the process and techniques of DNA extraction and analysis, and 2) to demonstrate the utility of DNA profiling.

Materials:

Inoculating needle
Broth
Culture tubes
Lysis buffer (50mM Tris-HCl, pH 8.0; 62.5mM EDTA, pH 9.0; 2.5M LiCl; Triton X-100 4%, v:v)
bacterial culture
Phenol:chloroform
Loading dye
Ethanol
Agarose gel
PCR enzyme mix
TBE
Primer sets
PCR tubes

Protocol

Bacterial inoculation

1. The day prior to your lab, each group needs to grow their bacterial culture in preparation for the lab.
2. Sterilely, add 2 mL of broth to a culture tube.
3. Again sterilely, scrape your frozen stock using the inoculating needle and swish in the broth.
4. Grow overnight at 37°C.

DNA Isolation (Week of September 9, 2023)

1. Harvest *E.coli* by centrifugation at 3000 rpm for 10 minutes. Wash once in 1 ml 1x PBS.
2. Resuspend cells in 150µL lysis buffer by sharply inverting the tube 3 times (DO NOT VORTEX OR SHAKE THE TUBE as this will shear the DNA).
3. Incubate 5 minutes at room temperature.

4. Add 150µL phenol:chloroform mixture and slowly shake tube by hand for 5 minutes.

IMPORTANT SAFETY NOTE: Phenol and chloroform and hazardous chemicals, wear gloves at all times and use in the fume hood.

5. Separate the phases by centrifugation at 7500 rpm for 5 minutes.
6. Collect the upper phase and transfer to a clean microcentrifuge tube.
7. Add 300µl of absolute ethanol to the new tube containing the organic phase to precipitate the nucleic acids.
8. Swirl the mixture gently for 15 seconds and then incubate at room temperature for 5 minutes.
9. Centrifuge at 8000 rpm for 10 minutes to collect the nucleic acids.
10. Remove the supernatant, making sure not to dispose of the DNA pellet.
11. Add 1 ml of absolute ethanol to wash the pellet. Mix well.
12. Centrifuge at 8000 rpm for 5 minutes.
13. Remove the supernatant, making sure not to dispose of the DNA pellet.
14. Briefly air-dry (~10minutes) and invert the tube over a kimwipe to finish drying.
15. Resuspend the DNA in 100 µl of TAE Buffer.
16. Store in refrigerator for next lab.

PCR Amplification (Week of September 23, 2024)

1. In a PCR tube add the following matrix:

DNA	Taq Master mix	Primers	Water	Total volume
1 µl	25 µl	1 µl each	22 µl	50 µl

2. The entire lab section will also prepare a standard control for the reaction.
3. Place in the PCR machine and run XXXX program. (TA will indicate program)
4. Store in freezer for next lab.

Gel Electrophoresis (Week of September 23, 2024)

1. Take a clean tube and combine 5 µl of your PCR product with 3 µl of loading dye. Mix well.
2. Place your agarose gel (0.5% agarose in TBE) in the electrophoresis chamber.

IMPORTANT SAFETY NOTE: The agarose gel contains EtBr which is an intercalating agent and can therefore be mutagenic and carcinogenic.

3. Add TBE to cover the gel. After your gel is completely immersed, load 1 lane with 100bp ladder. You should also load your samples from step 1 in the remaining lanes.
4. Run the gel at 100v for 40 minutes.
5. Visualize by UV light.

IMPORTANT SAFETY NOTE: Do not look directly at the UV light as it is mutagenic and can cause serious burns.

6. Record your results compared to the loading standard determining the size of your PCR band.

Lab Report: (address the following)

Why are you doing this lab?
How did you do this lab?
What were the results of this lab?
What do the results mean?
Where there any problems during the lab/with the results?
How can these results help to discover the identity of your unknown bacterium?

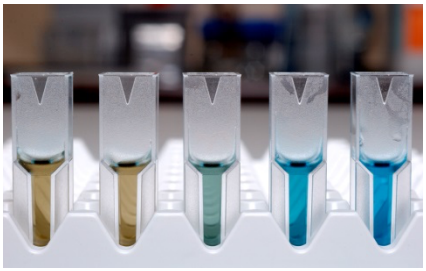
Microbial Physiology Lab
Fall 2021

Protein Extraction and Bradford assay
Lab 5 – Week of September 30, 2024

Introduction

Because each bacterial species has a unique physiology, they express different proteins at different levels. By quantifying which proteins are expressed and at what levels, one can therefore differentiate between bacterium. In this lab you will isolate bacterial proteins and quantifying them in preparation for identifying variable expression of these proteins between bacterial species.

There are numerous methods to quantify total protein; you will use a fast and simple method called the Bradford assay. The Bradford assay is a colorimetric procedure for determining the concentration of total solubilized protein. It utilizes a colorimetric, acidic dye, Coomassie blue, which is added to a protein containing solution. The dye binds to proteins in the solution, mostly to basic and aromatic amino acids, and changes color. This color change is detected with a spectrophotometer. Normally, Coomassie blue has an absorption peak at 650 nm giving it a green-yellow color (far left in the figure). Upon bind to proteins, its absorption peak shifts to 595 nm resulting in a deep blue color (far right in the figure). A more intense blue color indicates the presence of more protein in a given sample. Mixtures of bound and unbound Coomassie blue result in the intermediate shades. The color can be quantified using the spectrophotometer to measure light absorbance.



Materials

Bacterial cultures
Lysis buffer (10 mM Tris-HCl pH 7.9, 1.5 mM $MgCl_2$, 0.3 M sucrose, 0.5% Triton X-100)
BioRad Bradford Assay reagent
Bovine serum albumin (standard)
Spectrophotometer

Protocol

Bacterial inoculation

1. The day prior to your lab, each group needs to grow their bacterial culture in preparation for the lab.
2. Sterilely, add 2 mL of broth to a culture tube.
3. Again sterilely, scrape your frozen stock using the inoculating needle and swish in the broth.
4. Grow overnight at 37°C.

Protein isolation

1. Harvest *E. coli* by centrifugation 3000 rpm for 8 minutes.
2. Remove and discard the supernatant.
3. Wash the cell pellet by resuspending it in 1 ml of PBS.

4. Centrifuge at 3000 rpm for 8 minutes.
5. Remove and discard the supernatant. Make sure not to disturb the pellet.
6. Resuspend the cell pellet in 200 µl of lysis buffer by sharply inverting the tube 3 times (do not vortex or shake the tube).
7. Incubate 5 minutes at room temperature.
8. Centrifuge at 8000 rpm for 10 minutes.
9. Collect the supernatant and transfer it to a new tube. This constitutes the whole cell extract. Discard pellet.

Protein quantification

1. Make a serial dilution of the BSA standard to include 2, 1.5, 1, 0.75, 0.5, 0.25 mg/mL.
2. Add 20 µl of whole cell extract or standard to the bottom of a cuvette. Make sure to add the entire volume.
3. Add 1 ml of BioRad protein reagent to the cuvette.
4. Read the samples in the spectrophotometer at 595 nm. Do not forget to include a blank sample (cuvette containing BioRad protein reagent only).
5. Record the results.

Lab Report: (address the following)

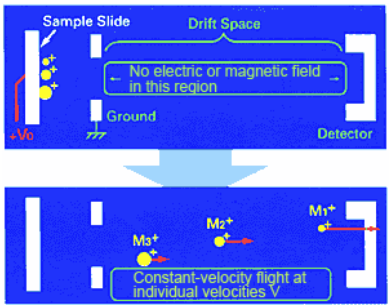
Why are you doing this lab?
How did you do this lab?
What were the results of this lab?
What do the results mean?
Where there any problems during the lab/with the results?
Why is knowing the protein concentration important?

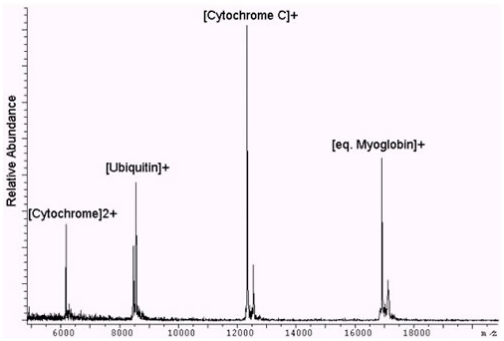
Microbial Physiology Lab
Fall 2021

Mass Spectrometry
Lab 6 – Week of October 7, 2024

Introduction

In this lab, you will use MALDI-TOF MS to help identify your unknown bacterium. MALDI is the abbreviation for Matrix Assisted Laser Desorption/Ionization. TOF MS is the abbreviation for Time of Flight Mass Spectrometry. You will mix your bacterial sample with a matrix and dry it onto a metal plate. The matrix absorbs laser light and converts it to heat energy. A small part of the matrix heats rapidly (in several nanoseconds) and is vaporized, together with the sample. Through absorption of the energy, these samples also obtain an ionization charge. These charged molecules are accelerated towards the detector. With no electric or magnetic field, ions with a smaller mass/unit charge (m/z) and more highly charged molecules will reach the detector before heavier molecules. Consequently, the time of ion flight differs according to the m/z value of the ion. Based upon the time of arrival of the ions, the mass/charge for each ion detected is calculated and displayed graphically. The accumulation of numerous m/z values increases the peak height for that m/z. Therefore, the peak height differences vary according to the relative abundance of each m/z ion within the sample.





MALDI-TOF spectra can be used for the identification of microorganisms such as bacteria or fungi. Since each bacterial species has a different collection of proteins in different abundances, MALDI-TOF analysis would result in slightly different mass spectrograms for different species. The mass spectrograms generated are analyzed by dedicated software and compared with stored profiles. Species diagnosis by this procedure is much faster, more accurate and cheaper than other procedures based on immunological or biochemical tests. MALDI-TOF may become the standard method for species identification in medical microbiological laboratories over the next few years.

Materials

Protein isolated from bacterial cultures last week
Matrix (Sinapinic Acid, reconstituted with 1mL of 2:1 ACN-Water 0.1% TFA)
2:1 Acetonitrile:Water with 0.1%Trifluoroacetic acid (TFA)
MALDI plate and spectrometer

Protocol

Sample Preparation

1. In a microcentrifuge tube add 5µL of 1000µg/mL of your protein sample and label this as “sample tube, group name”
2. Add 2.5µL of matrix to your sample tube and mix by carefully pipetting up and down
3. Add 1.5µL of 2:1 acetonitrile:water solution with 0.1%TFA to your sample tube and carefully mix with pipette.
4. Take 1µL of your sample and plate onto the metal MALDI plate.
   - **Take note of where you positioned your sample ⬇**

   _____
5. Allow to dry completely.
6. Place plate in the mass spectrometer.

Analyzing Data on the MALDI-TOF Mass Spectrometer

1. Turn on Computer and make sure MALDI is also on
2. Select program “Shimadzu Biotech MALDI-MS”
3. Select “Instrument” and “Acquisition”
4. When window opens, select tap “Exp. Tech.” and click “Open Door” and select Yes
5. *Wear gloves to grab plate from door*
6. Remove plate and place on surface and click “Close Door”
7. Spot samples of plate
8. Allow the sample to air dry
9. Again, “Open Door” and place plate inside and “Close Door”
10. After opening the plate door and inserting the plate, allow the pressure to increase until instrument status indicates that it is OK to proceed with sample analysis
11. Select “View” on first window and “Instrument Status”
12. Wait until “Turbo 1” and “Laser” on picture turn Green
13. In Acquisition window, select tab “Firing”
14. Shots: 100
15. Select well on Yellow picture (ONE WELL AT A TIME)
16. Under Auto Quality tab type mass range (30,000-300,000)
17. Click FIRE
18. Save under desired file name
19. Results will be displayed on first window when Acquiring profile reaches 20 (bottom right of Acquisition window)
20. Add cursors by clicking on scroll wheel and placing them with the peak in the middle
21. Right click and add peak – mass should appear
22. Delete cursors on right hand of screen where a cursor off button appears
23. To save results, select “Edit”, “Copy Window”, “As a Metafile” and paste into a Powerpoint slide.

24. Compare the spectrum for your unknown sample to the six control samples. Align the peaks to determine the most likely candidate for your unknown.

Lab report questions

1. Why are you doing this lab?
2. How did you do this lab? What ionization method are we using? How does it work?
3. What were the results of this lab?
4. What do the results mean?
5. Where there any problems during the lab/with the results?
6. What evidence supports your identification of your unknown bacterium?
7. What differences exist between your sample and the known samples? What do they mean? Are they important?

Microbial physiology lab
Fall 2024

Lab Midterm

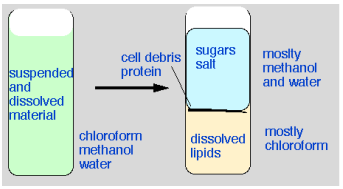
Week of October 14, 2024

Microbial physiology lab
Fall 2024

Lipid Extraction & Thin Layer Chromatography (TLC)
Lab 7 – Week of October 21, 2024

Introduction

Unlike the DNA you extracted previously (and proteins that you threw away), lipids cannot be extracted in aqueous solutions due to their hydrophobic tails. Therefore, organic solutions such as chloroform ($CHCl_3$) are used to extract lipids. *IMPORTANT SAFETY NOTE: Chloroform is an anesthetic. Its vapors depress the central nervous system and can result in cardiac or respiratory arrest. Use only in the fume hood and do NOT inhale the vapors.* The hydrophobic tails of lipids will intercalate with the chloroform dissolving most lipids in the chloroform (with some water associated with the polar heads). Conversely, the highly hydrophilic proteins, sugars and salts will be dissolved in the methanol and water. Following centrifugation, the two immiscible solvents will separate to form a discontinuous mixture with the aqueous portion on top and the heavier chloroform on the bottom. One can then separately obtain the lipids verses the sugars/proteins.



There are many different types of lipids. The following protocol will extract most of these; however, more specialized protocols exist for extracting specific categories of lipids, e.g., cholesterol. Since this protocol extracts many different types of lipids, they would need to be subsequently separated by extraction with various combinations of organic and aqueous solutions. Because each bacteria has a unique set of gene-encoding metabolic enzymes, different bacterial species synthesizes different combinations of lipids and other metabolic intermediates. This analysis of unique combinations of metabolism products is termed metabolomics. By extracting and analyzing these lipids, one can identify an unknown bacterial species.

Materials

E. coli culture
Chloroform:Methanol:water (1:2:0.8)
Chloroform:Methanol (2:1)

Protocol

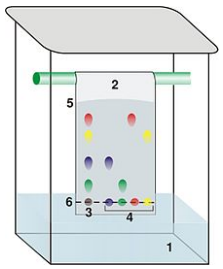
1. Harvest 10 ml *E. coli* by centrifugation 3,000 rpm for 8 minutes.
2. Resuspend the cell pellet in 1ml PBS and centrifuge again. Make sure to eliminate all traces of liquid.
3. Resuspend the pellet in 1ml chloroform: methanol: water (1:2:0.8).
4. Vortex or shake well for 1 min.
5. Centrifuge tube at 10,000 rpm for 5 min at room temperature.
6. Remove the organic phase and transfer to a clean tube.
7. To the first tube, add 1ml chloroform : methanol (2:1)
8. Centrifuge tube at 10,000 rpm for 5 min at room temperature.
9. Remove the organic phase and transfer it to the previously extracted organic phase.
10. Freeze at -20C immediately and save for next lab, Thin Layer Chromatography.

Thin Layer Chromatography

Introduction

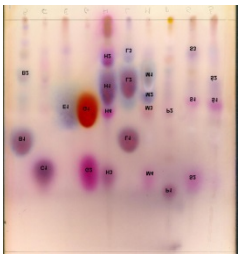
Thin layer chromatography (TLC) is a simple, quick, and inexpensive procedure to show how many components are in a mixture. TLC can also be used to support the identity of an unknown compound in a mixture by comparing the retention factor (R_f) of the unknown compound with the R_f of known compounds (preferably both run on the same TLC plate). Therefore, TLC can serve many purposes and has applications in biochemistry, organic chemistry, forensics, food monitoring, and pharmaceutical analysis.

A TLC plate is a sheet of glass, metal, or plastic which is coated with a thin layer of a solid adsorbent (usually silica or alumina). This layer of adsorbent is the stationary phase (similar to agarose for DNA electrophoresis). A small amount of the mixture to be analyzed is spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the very bottom of the plate is in the liquid. This liquid, or the eluent, is the mobile phase and slowly rises up the TLC plate by capillary action.



As the solvent rises up through the adsorbent, differential partitioning occurs between the components of the mixture dissolved in the solvent and the stationary adsorbent phase. The more strongly a given component of a mixture is adsorbed onto the stationary phase, the less time it will spend in the mobile phase and the more slowly it will migrate up the plate.

When the solvent has reached the top of the plate, the plate is removed from the developing chamber, dried, and the separated components of the mixture are visualized. If the compounds are colored, visualization is straightforward. Usually the compounds are colorless, so a UV lamp is used to visualize the plates. The plate itself contains a molecule which fluoresces everywhere *except* where an organic compound is present.



Materials

TLC plates
Sample
Eluent (120mL chloroform, 60mL acetone, 52mL methanol, 48mL acetic acid, 28mL DDI H_20)

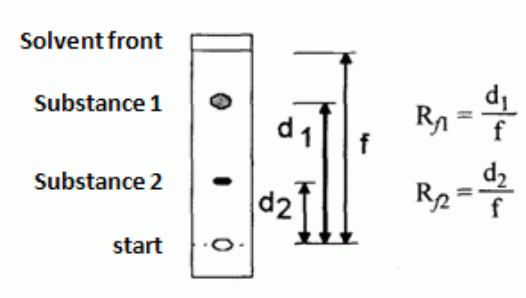
Protocol

1. There will be 2 plates for the entire group. Be careful not to scratch the surface of the plate. Wear gloves when handling the TLC plate.
2. With a pencil (preferably one with a dull point), draw a line 1 inch from the top of the plate and label your plate with the section and team numbers.
3. Draw up 10 µL of sample and dot a small amount onto one of the lanes at the bottom of the plate. Do not press the button on the pipette; use capillary action to dot the sample on. Wait for the spot to dry completely before placing another 10 µL drop directly on top of the first. Your sample should be concentrated to as small a spot as possible. Repeat until 40 ul has been spotted.

4. Line your TLC chamber with filter paper and fill the chamber with the eluent. Douse the filter paper with the same eluent to create a humid environment.
5. Place your plate upright in the chamber and allow it to process for 1 hour, or until the liquid has traveled up to the line at the top of the plate.
6. Allow your plate to dry (~10 minutes).
7. Place the dried TLC plate in the iodine chamber for 3-4 days.

**When handling the eluent, be careful to cap your bottle or chamber right away to prevent the chloroform from evaporating.

Analysis
The components, visible as separated spots, are identified by comparing the distances they have traveled with those of the known reference materials. Measure the distance of the start line to the solvent front. Then measure the distance of center of the spot to the start line. Divide the distance the solvent moved by the distance the individual spot moved. The resulting ratio is called R_f-value (retention factor). The value should be between 0.0 (spot did not moved from starting line) and 1.0 (spot moved with solvent front) and has no units.



$$Rf = \frac{\text{the distance from the starting point to the center of the spot}}{\text{the distance from the starting point to the solvent front}}$$

Lab Report: (address the following)
Why are you doing this lab?
How did you do this lab?
What were the results of this lab?
What do the results mean?
Where there any problems during the lab/with the results?
How did your sample compare to the reference material?
How could you use these results to discover the identity of an unknown bacterium?

Microbial physiology lab
Fall 2024

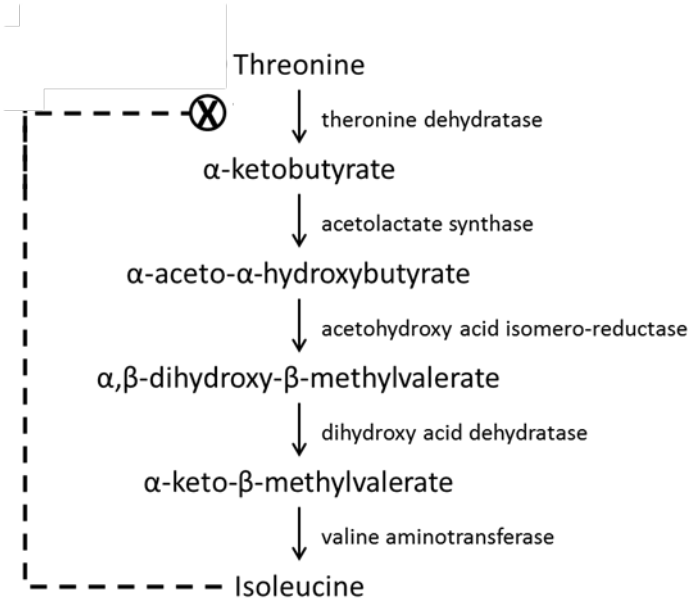
Feedback Inhibition
Lab 8 – Week of October 28, 2024

Feedback Inhibition

The term feedback inhibition refers to a biochemical reaction in which the products generated at the end of a series of enzymatic reactions inhibit a reaction at the beginning of the cascade. In feedback inhibition, there is a second binding site on the enzyme where the inhibitor binds, so that the inhibitor is not necessarily similar in structure to the substrate.

The absence or presence of the inhibitor at this second binding site activates or deactivates the enzyme, presumably by changing the conformation of the enzyme so that the active site is made available or unavailable to the substrate. The inhibitor is usually the product of a reaction farther on down the metabolic pathway.

The diagram shown below schematically depicts a series of five reactions, each catalyzed by its own enzyme, which converts L-threonine to L-isoleucine. Enzyme one, which catalyzes the reaction which involves the removal of the amino group from L-threonine, is called L-threonine dehydratase. This enzyme is strongly inhibited by the ultimate product of the five reactions, L-isoleucine. L-isoleucine is quite specific as an inhibitor; other amino acids or related compounds do not inhibit that enzyme. In this way the cell regulates the amount of isoleucine produced. When the concentration of isoleucine begins to get high, the whole chain of reactions is shut down by the inhibition of the first reaction in the series.



Watch this video: https://www.youtube.com/watch?v=qHb7iieM2Ro

The object of this experiment is to determine the feedback inhibition regulation of this pathway. You have the following starting materials:

1. A mix containing all 5 enzymes
2. α-ketobutyrate
3. α-acteo-α-hydroxybutyrate
4. α,β-dihydroxy-β-methylvalerate
5. α-ket-β-methylvalerate
6. Threonine
7. Isoleucine
8. Leucine
9. Valine
10. A mechanism by which to measure the activity of valine aminotransferase

Design an experimental setup in order to demonstrate feedback inhibition in this pathway and its specificity (don't worry about timing). Create a graph demonstrating the hypothetical data and interpret your findings. Use as many controls as necessary to get accurate interpretable data.

Microbial physiology lab
Fall 2023

Fermentation Part 1 & 2
Lab 9– Week of November 4, 2024

Introduction

Fermentation is a metabolic process, by which cells are able to produce energy using organic compounds such as carbohydrates. This biochemical process uses an organic compound as an electron donor, in contrast to respiration, in which the electron donors are inorganic (oxygen). The most common substrates for fermentation are sugars such as glucose, fructose, and sucrose. Other compounds such as butyric acid and acetate can also serve as substrates.

Fermentation, just like cellular respiration, is preceded by glycolysis, a series of biochemical reactions that break a 6 carbon carbohydrate into two 3 carbon molecules of pyruvic acid. It is important to remember that glycolysis occurs in the cytoplasm in bacteria, not in some specialized organelle. In cellular respiration, after glycolysis takes place, the resulting molecules of pyruvic acid enter the Krebs cycle, followed by the mitochondrial electron transport chain. The pyruvic acid molecules are completely broken down to CO_2 and more energy is produced. In fermentation, after glycolysis has generated two pyruvic acid molecules, these are turned into cellular energy (in the form of ATP) and some waste product, usually CO_2 and ethanol, are formed. Fermentation can also occur in animal cells, specifically in muscle cells. In the absence of oxygen (a condition that can occur during exercise), muscle cells are able to produce energy by turning pyruvic acid into lactic acid. Since muscle cells cannot use the lactic acid, it is up to the bloodstream to gradually transport it to the liver, where it is metabolized.

Fermentation has many applications in our daily lives: production of bread, alcoholic beverages, yogurt, vinegar, and fuel. The discipline that studied fermentation is known as zymology. The two most widely known fermentations are ethanol and lactic acid fermentation. Ethanol fermentation is performed by yeast such as *Saccharomyces cerevisiase* and some bacteria such as *Zymomonas*. The waste products of this fermentation are ethanol and CO_2. The general reaction for this type of fermentation is as follows:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

Lactic acid fermentation is performed by some fungi and bacteria such as *Lactobacillus*. It can also occur in muscle cells. Lactic acid fermentation can be homolactic, if two molecules of lactic acid are produced, and heterolactic, if one molecule of lactic is produced along with other molecules. The general reaction for homolactic fermentation is as follows:

$$C_6H_{12}O_6 \rightarrow 2C_3H_6O_3$$

On the other hand, the reaction for heterolactic fermentation with CO_2 and ethanol is:

$$C_6H_{12}O_6 \rightarrow C_3H_6O_3 + CO_2 + C_2H_5OH$$

The products of heterolactic fermentation vary based upon the enzymes bacterial species express. Therefore, analysis of the products of fermentation can be utilized to rule out possibilities of an unknown bacterium.

Part 1 (Week of November 4, 2024)

Materials
Milk
Yogurt
Beer kit
Hydrometer
Reflectometer & test strips

Protocol

Yogurt production

1. Dispense 50 ml of milk into 2 sterile beakers/flasks.
2. Label 1 beaker/flask as control and the other one as experimental.
3. To the experimental beaker/flask, add 2 ml of yogurt and mix thoroughly by shaking. Do not add yogurt to the control beaker/flask.
4. Incubate in a water bath at 45 ^{o}C for 1 hour.
5. Refrigerate until next lab period.

Beer production

Protocol (Muntons beer-making kit)
1. Dispense the contents of the Muntons can (containing malt) into the glass container with warm sterile water.
2. Add sugar (sucrose) to the mixture and mix thoroughly.
3. Remove 100 ml of the mixture as the starting solution.
3. Use the hydrometer to measure the specific gravity of the starting solution.
4. Add the brewer's yeast.
5. Thoroughly mix the container.
6. Cover fermenter and keep it until next lab period at room temperature.

Microbial physiology lab
Fall 2024

Fermentation Part 2

Introduction

This is the continuation of the Fermentation lab. See page 32 for Introduction.

Part 2 (Week of November 4, 2024)

1. Dip 1 test strip into the control yogurt culture, wipe it clean and insert it into the reflectometer.
2. Dip 1 test strip into the experimental yogurt culture, wipe it clean and insert it into the reflectometer.

3. Use the hydrometer to measure the amount of ethanol in the fermented solution.
4. Go to http://www.brewersfriend.com/abv-calculator/ to calculate the amount of alcohol produced during fermentation.

Lab Report: (address the following)

Why are you doing this lab?
How did you do this lab?
What were the results of this lab?
What do the results mean?
Where there any problems during the lab/with the results?

Microbial physiology lab
Fall 2024

Lab Final

Week of November 18, 2024