General Microbiology Laboratory

Lab Session 8 - Microbial Genetics

Introduction

Procaryotic information, and the nature of information flow according to the Central Dogma, is essentially identical to eucaryotic systems. Additionally, microorganisms are subject to mutations analogous to eucaryotes. Not surprisingly, procaryotes have many types of mutation repair mechanisms. Mutations that are not repaired are heritable, and can be easily recognized if the mutation results in a phenotypic change. The purpose of this lab is to investigate phenotypic variation in microorganisms, in particular how phenotype and survivability is affected by mutations and mutation repair.

A. Review the following for reference:

1. Lab Manual:
   Exercise #21 - Pages 130 and 131. Read for prep of gradient plates.
   Exercise #23 - Pages 143 and 144. Read for background only.
   Exercise #27 - Page 165 and 167. Read for background and preparation of "spread plate.
2. Text:
   Chapter 7 - Page 141. Review only!
   Chapter 13 - Pages 317-323 and 326-329. Review only!
3. Pay particular attention to the following:
   -Techniques used in preparing gradient plates.
   -General information on mutations.
   -General information on effects of UV light on mo's

B. Do the following during lab:

1. Phenotypic Variation: Effect of Temperature on Phenotype
   a. Prepare: 3 streak plates (using inoculating loop) of *Serratia marcescens* and incubate each for 48 hr as follows:
      #1 - At 23 C
      #2 - At 30 C
      #3 - At 37 C
   b. Results: Describe and explain any differences you observe.

   Note: -Label all cultures with Group/Table number, and identify well.
   -Incubate cultures in incubators/drawers so that all materials can be easily found later.

2. Spontaneous Mutations: Development of Resistance to Streptomycin
   2.1. Gradient Plate Technique.
   a. Prepare: 6 gradient plates as follows:
      -Pour 15 ml of melted agar WITHOUT streptomycin into each of 6 petri dishes.
      -Place at an angle and allow them to solidify. Don't disturb the agar as it hardens.
      -Pour 15 ml of melted agar WITH streptomycin into petri dishes as follows:
      (2 plates/concentration):
      #1 A & B - 0.005 mg/ml
      #2 A & B - 0.01 mg/ml
      #3 A & B - 0.03 mg/ml
      -Leave the dishes flat; mark the point of highest streptomycin concentration and draw a line to the point of lowest concentration; allow these to solidify; put in refrigerator for 3-5 minutes to thoroughly harden.
      -Inoculate set 'A' with *E. coli* and *S. marcescens* (with sterile loops) as demonstrated. [Each mo will be inoculated on each plate along the concentration gradient.]
      -Incubate at 30 C for 24-48 hr.
   b. Repeat: Use a loop to choose a colony of the organism growing at a high concentration of antibiotic and transfer it to a new (i.e. set 'B') gradient plate of the same concentration.
   c. Results: Draw, describe and explain the growth patterns.

2.2. Dilution Technique.

a. Prepare: 2 sets of 10 tubes of TSB as follows:

-Add 25 ul of *S. marcescens* or *E. coli* (using micropipet) to each of one set of 10 tubes of TSB containing the following concentrations of streptomycin:

#1A -	0.0 mg/ml	#6A -	0.1 mg/ml
#2A -	0.01 mg/ml	#7A -	0.3 mg/ml
#3A -	0.03 mg/ml	#8A -	0.5 mg/ml
#4A -	0.05 mg/ml	#9A -	0.75 mg/ml
#5A -	0.075 mg/ml	#10A -	1.0 mg/ml

-Incubate at 30 C for 24-48 hours

b. Repeat: Take the tube with the highest concentration of antibiotic that has growth, and transfer 25 ul aliquots to the second series of tubes with streptomycin (Tubes #1B to #10B).

c. Results: Note presence or absence of growth in each of the two sets of tubes.

3. Induced Mutations: Mutagenic and Lethal Effects of UV Light and Chemical Mutagens

3.1. Mutagenic and Lethal Effects of UV Light

a. Prepare 2 sets of 10 TSA plates as follows:

-Add 10 ul *S. marcescens* or *B. cereus* or *E. coli* (as directed) to each of 10 TSA plates.

-Add 10 ul *S. marcescens* or *B. cereus* or *E. coli* (as directed) to the 2nd set of 10 TSA plates.

-Spread evenly over the surface of each plate (as demonstrated) using a sterile swab.

-After the inoculum dries! expose the surface of the plate with UV light for the following times:

#1 - 0 sec	#6 - 90 sec
#2 - 10 sec	#7 - 120 sec
#3 - 20 sec	#8 - 180 sec
#4 - 40 sec	#9 - 240 sec
#5 - 60 sec	#10 - 360 sec

-Incubate at 30 C for 24-48 hours

b. Results: Note density of growth (i.e. the number of colonies, not the size of the colonies) as a function of time of treatment with UV light

3.2. Mutagenic and Lethal Effects of Chemical Mutagens

Skip.

4. Mutation Repair: Photoreactivation

a. Prepare: 2 sets of 10 plates as follows:

-Add 10 ul *S. marcescens* to one set and 10 ul *E. coli* to the second set of TSA plates;

-Spread evenly over the surface of each plate with a swab and allow inoculum to dry!

-Expose the plates to UV light for the following time periods (either 20, 30, 45 or 60 sec) and then incubate (under lamp or in desk drawer [NOTE: You must place the cultures to be incubated in the dark in your lab drawer immediately!!!] as follows:

#	Exposure	Incubation	#	Exposure	Incubation
1D	0 Sec	Dark	1L	0 sec	Light
2D	15 sec	Dark	2L	15 sec	Light
3D	30 sec	Dark	3L	30 sec	Light
4D	45 sec	Dark	4L	45 sec	Light
5D	60 sec	Dark	5L	60 sec	Light

b. Results: Note effects of UV light and effects of incubation in light or dark on growth.

C. Techniques: Preparation of Gradient Plates and Spread Plates

-Pour 15 ml of melted agar WITHOUT streptomycin into petri dish.

-Place at an angle and allow them to solidify. Refrigerate for 5 minutes.

-Pour 15 ml of melted agar WITH streptomycin into dish, mark the gradient.

-Leave the dishes flat, allow them to solidify, refrigerate 5 minutes..

D. Results:

1. Phenotypic Variation. Describe phenotype:

Temperature	Growth
23 C	
30 C	
37 C	

2.1. Spontaneous Mutations: Gradient Plate Technique. Draw/describe growth patterns:

Set 'A'

0.005 mg/ml	0.01 mg/ml	0.03 mg/ml

Set 'B'

0.005 mg/ml	0.01 mg/ml	0.03 mg/ml

2.2. Spontaneous Mutations: Dilution Technique: Describe growth:

Tube#	Concentration	*E coli* Set 'A'	*E coli* Set 'B'	*S. marcescens* Set 'A'	*S. marcescens* Set 'B'
#1 -	0.0 mg/ml				
#2 -	0.01 mg/ml				
#3 -	0.03 mg/ml				
#4 -	0.05 mg/ml				
#5 -	0.075 mg/ml				
#6 -	0.1 mg/ml				
#7 -	0.3 mg/ml				
#8 -	0.5 mg/ml				
#9 -	0.75 mg/ml				
#10 -	1.0 mg/ml				

3.1. Induced Mutations: Effects of UV Light. Describe density of growth:

Plate#	Time	MO #1	MO #2
#1 -	0 sec		
#2 -	10 sec		
#3 -	20 sec		
#4 -	40 sec		
#5 -	60 sec		
#6 -	90 sec		
#7 -	120 sec		
#8 -	180 sec		
#9 -	240 sec		
#10-	360 sec		

4. Photoreactivation. Describe growth patterns:

Plate	Time	Incubation	*S. marcescenes*	*E. coli*
#1D -	0 sec	Dark		
#1L -	0 sec	Light		
#2D -	15 sec	Dark		
#2L -	15 sec	Light		
#3D -	30 sec	Dark		
#3L -	30 sec	Light		
#4D -	45 sec	Dark		
#4L -	45 sec	Light		
# 5D -	60 sec	Dark		
#5L -	60 sec	Light		

E. Discussion: