

Microbiology for the Health Sciences Laboratory

Lab Session 8 - Microbial Genetics

Introduction

Prokaryotic information, and the nature of information flow according to the Central Dogma, is essentially identical to eukaryotic systems. Additionally, microorganisms are subject to mutations analogous to eukaryotes. Not surprisingly, prokaryotes 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.

A. Review the following for reference:

1. Text: Chapter 8 - Pages 188-197.
2. Pay particular attention to the following:
 - Techniques used in preparing gradient plates
 - General information on mutations.
 - General information on effects of UV light on m.o.'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:
 - # 1.1 - At 23 C
 - # 1.2 - At 30 C
 - # 1.3 - At 37 C
 - b. Record: Describe and explain any differences you observe.
2. Spontaneous Mutations: Development of Resistance to Streptomycin
 - a. Prepare: 3 gradient plates (see 'Techniques' for a picture of this technique) as follows:
 - Pour 15 ml of melted agar WITHOUT streptomycin into each of 3 petri dishes.
 - Place at an angle and allow them to solidify.
 - Pour 15 ml of melted agar WITH streptomycin into each of 3 petri dishes as follows:
 - # 2.1 - 0.005 mg/ml streptomycin
 - # 2.2 - 0.01 mg/ml streptomycin
 - # 2.3 - 0.03 mg/ml streptomycin
 - Leave the dishes flat; mark the area of highest streptomycin concentration; allow these to solidify; put in refrigerator.
 - Inoculate each with *E. coli* and *Serratia marcescens* (with sterile loops) as demonstrated.
 - Incubate at 23 C for 48 hr.
 - b. Record: Describe and explain the growth pattern.
3. Induced Mutations: Mutagenic and Lethal Effects of UV Light
 - a. Prepare: Lawns of growth on 8 plates as follows:
 - Add 0.01 ml (=10ul) of *S. marcescens* to each of 8 TSA plates:
 - Spread evenly over the outlined surface of each plate (as demonstrated) using a sterile swab.
 - After the inoculum dries!! expose the surface of each plate with UV light for the following times:
 - # 3.1 - 0 seconds
 - # 3.2 - 15 seconds
 - # 3.3 - 30 seconds
 - # 3.4 - 45 seconds
 - # 3.5 - 60 seconds
 - # 3.6 - 120 seconds
 - # 3.7 - 240 seconds
 - # 3.8 - 360 seconds
 - Incubate at 23 C for 48 hours
 - b. Record: Note density of growth as a function of time of treatment with UV light
 - c. Note: *Bacillus subtilis*, *Bacillus megaterium*, or *E. coli* may be substituted for *S. marcescens*.

4. Mutation Repair: Photoreactivation

a. Prepare: Lawns of growth on 8 plates as follows:

- Add 10ul (=0.01ml) of *S. marcescens* or *E. coli* to 8 TSA plates;
- Spread evenly over the outlined surface of each plate with a swab and allow inoculum to dry!
- Expose the plates to UV light for the following time periods (either 15, 30 or 45 sec) and then incubate in light (under lamp) or dark (in desk drawer) as follows:

Note: You must place the cultures to be incubated in the dark in your lab drawer immediately!!!

#	Exposure	Incubation	#	Exposure	Incubation
4.1	0 Sec	Dark	4.2	0 sec	Light
4.3	15 sec	Dark	4.4	15 sec	Light
4.5	30 sec	Dark	4.6	30 sec	Light
4.7	45 sec	Dark	4.8	45 sec	Light

b. Record: Note effects of UV light and incubation in light or dark

C. Techniques:

Preparation of Gradient Plates

- Pour 15 ml of melted agar WITHOUT streptomycin into petri dishes.
- Place at an angle and allow them to solidify. Refrigerate for 5 minutes.
- Pour 15 ml of melted agar WITH streptomycin into dishes, mark the gradient.
- Leave the dishes flat, allow them to solidify, refrigerate 5 minutes..

D. Results:

1. Phenotypic Variation. Describe phenotype for *Serratia marcescens*:

#1.1 - 23 C

#1.2 - 30 C

#1.3 - 37 C

2. Spontaneous Mutations. Draw and describe growth patterns for *Serratia marcescens* & *E. coli*:

#2.1 - 0.005 mg/ml

#2.2 - 0.01 mg/ml

#2.3 - 0.03 mg/ml

3. Induced Mutations. Describe density of growth for _____:

#3.1 - 0 sec

#3.2 - 15 sec

#3.3 - 30 sec

#3.4 - 45 sec

#3.5 - 60 sec

#3.6 - 120 sec

#3.7 - 240 sec

#3.8 - 360 sec

4. Photoreactivation. Describe growth patterns for _____:

#4.1 - 0 sec/dark

#4.2 - 0 sec/light

#4.3 - 15 sec/dark

#4.4 - 15 sec/light

#4.5 - 30 sec/dark

#4.6 - 30 sec/light

#4.7 - 45 sec/dark

#4.8 - 45 sec/light

E. Discussion: