

# Experimental evolution of antibiotic resistance in bacteria

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## 1 Abstract

We have designed a simple laboratory experiment that allows students in introductory biology courses, to observe the common soil bacterium, *Bacillus thuringiensis*, evolve resistance to the antibiotic streptomycin. This exercise allows students to observe and study evolution occurring in real time and to learn about the mechanisms underlying the current crisis in antibiotic resistance. An article that describes this exercise is in press (Krist & Showsh, in press). Copies of this poster are available on Amy Krist's website.

## 2 Introduction

Despite most respondents claiming some knowledge of evolution, nearly half of Americans considered themselves creationists in a 2001 Gallup poll (Alters & Nelson, 2002). Why do a majority of Americans reject one of the cornerstones of biology? Certainly the rise of Biblical literalism plays a role. However, misconceptions about evolution and the processes that cause it, contribute to the pattern. One way to address the disconnect between science and popular understanding is for students to "witness" the outcome of evolution, first hand, and to observe the operation of some of its causes.

We have developed a laboratory for introductory biology courses that allows students to observe evolution directly. In the lab, populations of a susceptible strain of the common soil bacterium, *Bacillus thuringiensis* evolve resistance to the antibiotic streptomycin. Students experimentally manipulate the environment of the bacteria to favor random mutations conferring resistance to streptomycin. To motivate the exercise, we place it in the context of understanding the current worldwide human health crisis in antibiotic resistance.

By using bacteria, students can observe changes on their own Petri plates. This personal observation helps to de-mystify the process of evolution by illustrating mutation and fitness variation. By placing the exercise in the context of a major human health crisis, we grab the students attention.

### 2.1 Objectives of the Laboratory

1. Observe a population of bacteria evolve.
2. Understand mutation and natural selection and their roles in the widespread phenomenon of antibiotic resistant bacteria.
3. Use evolutionary terminology to describe what occurred and how it relates to a "real-world" situation.

### 2.2 Bacteria and mutations

*Bacillus thuringiensis* obtains resistance to streptomycin by two different spontaneous mutations. One mutation alters ribosomal protein S12, the target for streptomycin binding, so that the mutated ribosome is still capable of synthesizing protein but is unable to bind to streptomycin (Snyder & Champness, 1997). Another mutation alters the shape of a protein in the cell membrane that is involved in transporting the antibiotic into the cell (Snyder & Champness, 1997). With a mutation rate of 1 per  $10^9$  generations applied and millions of cells that each

replicate over 16 million times during the course of the lab, mutations to streptomycin resistance occur on most students plates.

## 3 Procedures

### 3.1 Overview

- **Demonstrate that bacteria are viable and susceptible:** incubate bacteria on plates with a) nutrient agar (positive control) and b) streptomycin (negative control).
- **Manipulate the environment to increase fitness of mutants:** incubate the bacteria on a gradient plate, and streak the bacteria onto the part of the plate with streptomycin. Only the bacteria with a mutation for streptomycin will grow.
- **Demonstrate that mutated bacteria now grow on streptomycin plates:** incubate the resistant colonies from the gradient plate onto a second streptomycin plate.
- **Show that the population of bacteria have evolved:** compare the first and second streptomycin plates.

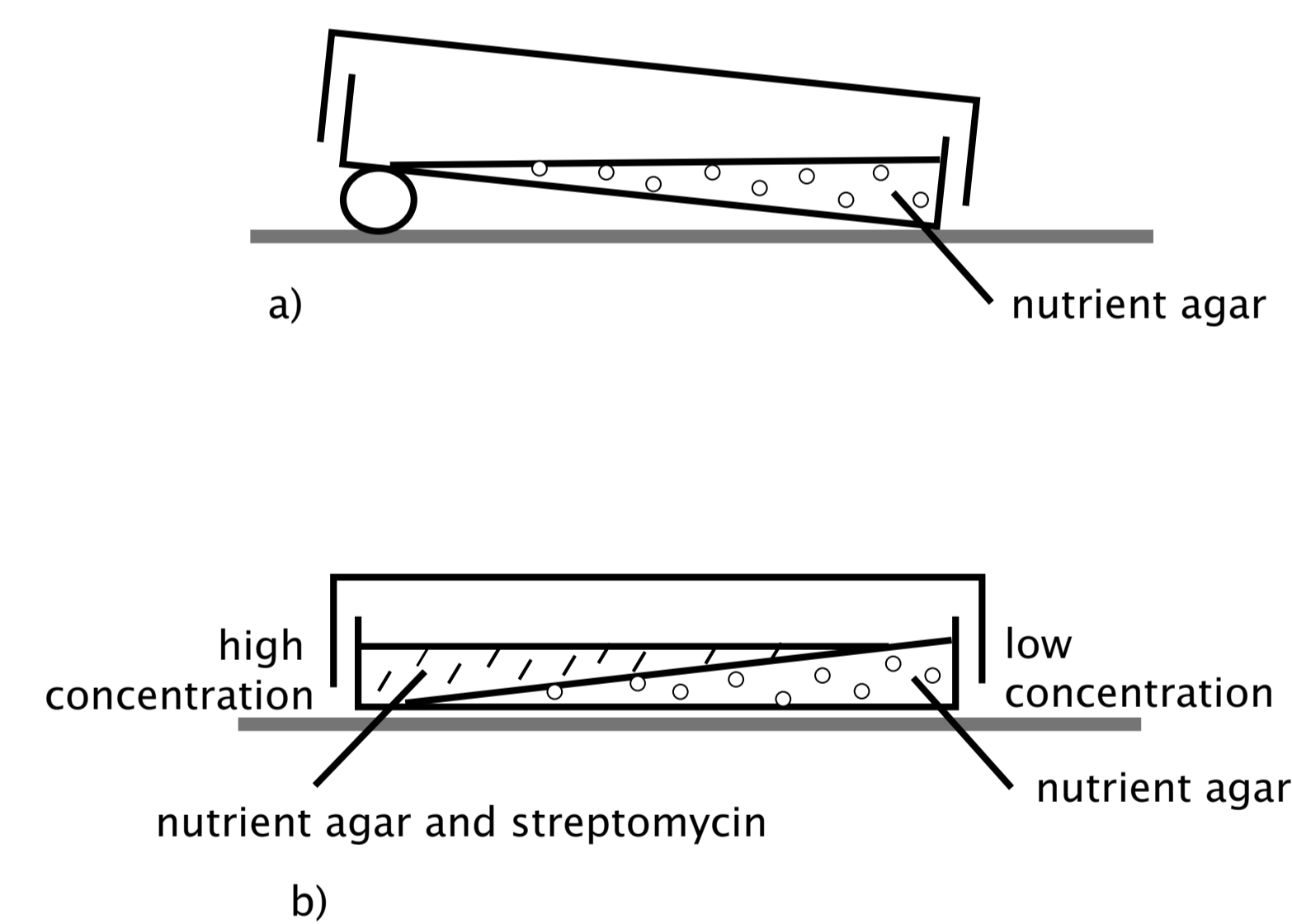


Figure 1. Preparation of an antibiotic gradient. a) Approximately 15 ml of nutrient agar is poured into a Petri plate, and allowed to solidify on a slant. We tip the plate up on a 10 ml pipette. b) 24–36 hours prior to use, the nutrient agar and streptomycin mixture is poured on top of the slant of nutrient agar. The entire surface is not covered so that one end of the plate contains only nutrient agar. High and low concentration refer to streptomycin.

### 3.2 Details

#### 3.2.1 First lab meeting: streak cells onto three plates

Students transfer bacterial cells to a nutrient agar plate and a streptomycin plate (Strep 1 plate). After incubation, growth on the nutrient agar plate demonstrates that the cells are viable and the absence of growth on the streptomycin plate demonstrates that they are susceptible to the antibiotic.

Students also streak bacteria onto a gradient plate which has a gradient of streptomycin from 0–250  $\mu\text{g}/\text{ml}$ . Refer to the legend for Fig. 1 for preparation of the gradient plate. Because diffusion will eventually eliminate the gradient, we do not pour the streptomycin agar onto the

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plate more than 36 hours before streaking. All plates are incubated, agar side up, for 24 hours at 37° C.

After streaking, students predict how the plates will appear after incubation and discuss a paper from the secondary literature. This paper addresses how antibiotics work and how antibiotics promote antibiotic resistance (Levy, 1998). The students prepare for the discussion by reading the paper in advance and writing a one-page summary and response.

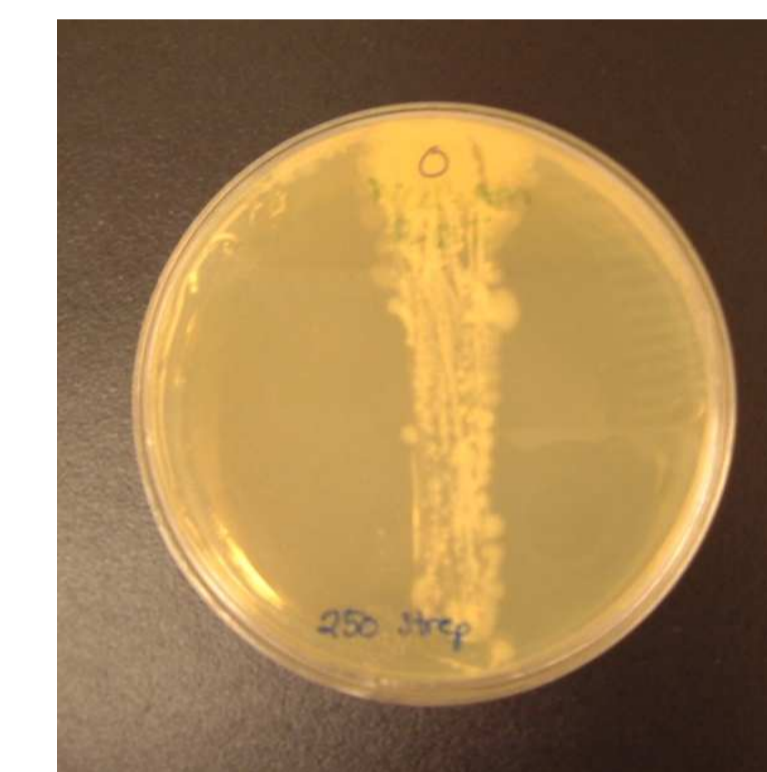


Figure 2. The appearance of the gradient plate after the plate was re-streaked twice and incubated at 37° C for 24 hours between each streaking. The colonies growing towards the bottom of the plate are resistant to streptomycin.

#### 3.2.2 Between the first and second lab meeting: re-streak gradient plates

Students or instructors re-streak the gradient plates one or two times between the first and second lab meetings. Re-streaking involves moving the bacteria growing on the part of the plate without streptomycin to the area of the plate with streptomycin. After each re-streak the plates are incubated for 24–48h at 37° C. If resistant colonies are not observed after the initial re-streak, the process can be repeated.

#### 3.2.3 Second lab meeting: transfer resistant colonies from gradient plate to Strep 2 plate

Students observe the plates and compare their predictions to the results. The gradient plate has been re-streaked one or more times (Fig. 2). If resistant colonies are present (most of the plates), the students transfer bacteria from these colonies to the Strep 2 plate and predict the outcome.

#### 3.2.4 Third lab meeting: wrap up, discuss results and literature, view film, and assessment

Students observe all of their plates and discuss the results. Specifically, we instruct the students to compare the positive control (nutrient agar plate), the negative control (Strep 1 plate), and the Strep plate with the resistant colonies (Strep 2 plate, Fig. 3). Working in groups, the students discuss why the plates look different, and the process that occurred on the gradient plate. After we discuss these topics as a class, the students watch a 20 min. segment from a recent film describing a strain of *Mycobacterium tuberculosis* that evolved resistance to multiple drugs in Russian prisons (excerpt from WGBH, 2001). After the film, we assess the students understanding of the lab and their ability to apply their knowledge to the novel situation that is presented in the film. In an in-class writing assignment, we ask the students a) to

use evolutionary terminology to explain why the exercise demonstrates evolution and natural selection, and how natural selection occurs in the experiment and b) describe an analogy between the situation portrayed in the film and the experiment.

## 4 Results & Discussion

After incubation, the nutrient agar plate has abundant growth of bacteria, the Strep 1 plate has no growth, and the gradient plate has abundant growth on the streptomycin-free part of the plate and no growth on the streptomycin. After re-streaking the gradient plate, most plates will have few to many resistant colonies (Fig. 2). Re-streaking increases the frequency of antibiotic resistant cells by moving resistant cells to the streptomycin part of the plate where they can grow without competing with susceptible cells. After a colony of resistant cells is growing in the streptomycin, re-streaking also physically separates resistant bacteria so that each cell can develop into a new colony.

The evolutionary processes occurring on the gradient plate are the random occurrence of mutations to antibiotic resistance and "natural" selection acting to increase the frequency of the mutants. Mutations to streptomycin resistance occur during division of bacteria on the streptomycin-free portion of the gradient plate. "Natural" selection occurs by simply moving cells from the streptomycin-free portion of the plate to the area with antibiotic; this increases the fitness of the mutant bacteria and decreases the fitness of the non-mutated cells. Hence, selection by streptomycin favors the antibiotic resistant bacteria.

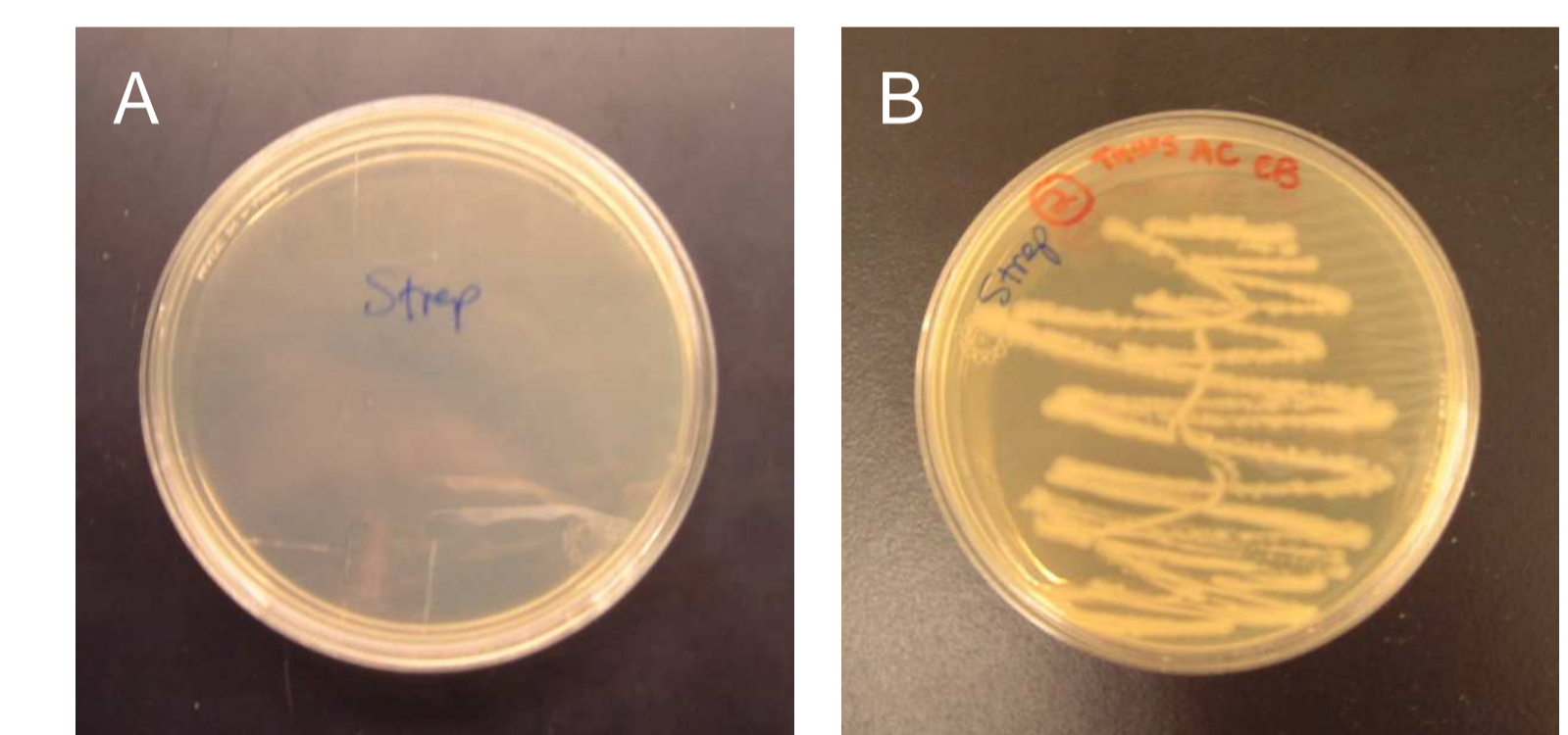


Figure 3. A) Strep 1 plate; the first streptomycin plate streaked with the initial culture of *B. thuringiensis* B) Strep 2 plate; the second streptomycin plate streaked with resistant colonies from the gradient plate. Comparison of these two plates shows that a population of *B. thuringiensis* evolved.

## References

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