MiSP Evolution / Bacterial Resistance Worksheet L1

Name _____ Date _____

EVOLUTION BY NATURAL SELECTION / BACTERIAL RESISTANCE WORKSHEET: NATURAL SELECTION OF TRICLOSAN-RESISTANT BACTERIA

Introduction:

Natural selection is a mechanism of evolution dependent on the environmental selection of organisms most fit to reproduce. This is sometimes called "survival of the fittest." An example of evolution by natural selection is the development of populations of bacteria that are resistant to antimicrobial agents as a result of exposure to these agents. Antimicrobials kill off susceptible members of a population, but cells that have some resistance through mutation or gene exchange may survive. These survivors are the "fittest" in that particular environment because they can survive and reproduce. In this activity you are provided with data that demonstrate the development of resistance of the bacterium *Escherichia coli* to the antimicrobial agent triclosan.

Triclosan is widely used as an antimicrobial agent (i.e., a substance toxic to bacteria, fungi and protists, and viruses). However, there is no real evidence that the addition of this agent to household products prevents infection in humans. On the other hand, several recent studies suggest that the overuse of triclosan-containing products can select for bacteria that are resistant to this chemical. Because many antimicrobial agents work by similar mechanisms, the development of resistance to triclosan may make bacteria resistant to other antimicrobials as well. For this reason, widespread use of triclosan may represent a potential public health risk with regard to development of resistance to clinically important antimicrobials.

In the experiment described here, the same disk-diffusion method that you used to study the inhibition of growth in *Bacillus cereus* was used to study the effects of triclosan on *Escherichia coli* (*E. coli),* a type of bacteria that can be harmful to humans. To test for the development of resistance, the researchers started with single colonies of bacteria from the fuzzy "halo" zone at the edge of the zone of inhibition of the original culture (called "culture 1"). The bacteria that grew in this area could grow in the presence of a concentration of triclosan that killed most of the other bacteria on the plate. The bacteria taken from the halo, therefore, represent mutant variants from the original population of bacteria that were plated in culture 1. The researchers spread these mutant bacteria on new agar plates. Ten of these bacterial plates were made; these plates were called "culture 2." A disk containing 0.1% triclosan was placed in the center of the plate. The bacteria were grown overnight, and the next day the diameter of the zone of inhibition was measured. Next, colonies from the edge of the zones of inhibition in culture 2 plates were used to make ten new plates, and these were also exposed to 0.1% triclosan using the disk-diffusion method. This was called "culture 3." In all, 12 separate cultures (ten plates for each culture) were made, and the diameters of the zones of inhibition were measured for each plate. The data that were collected can be found in table 1 below.

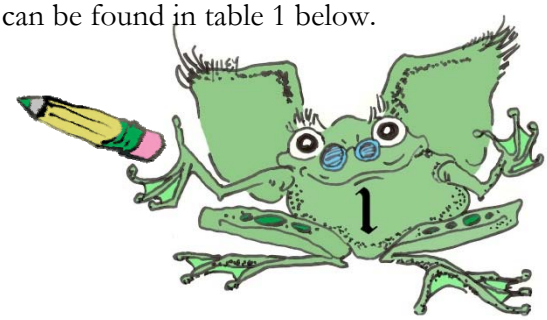
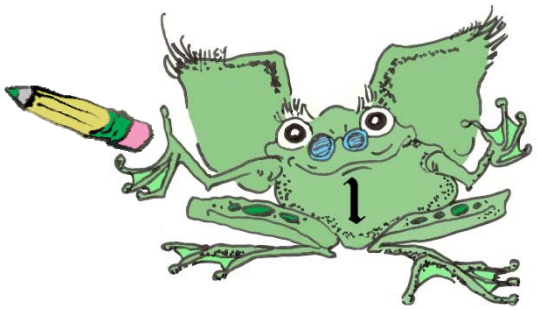


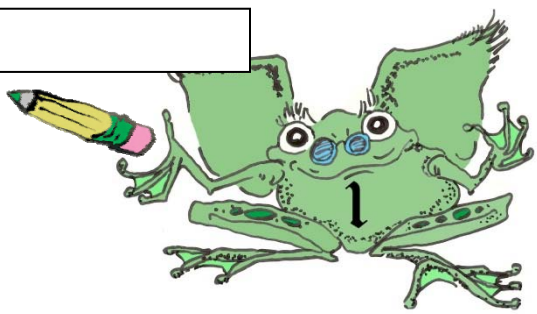
Table 1. Diameters of the zones of inhibition (mm) in ten individual plates and average diameter of the zone of inhibition (mm) for 12 separate cultures.

Plate #	Culture number											
	1	2	3	4	5	6	7	8	9	10	11	12
1	18	7.5	4	3.5	2.8	3	1.8	1.6	1.5	1.6	0.8	1.6
2	21.5	6.5	5	3	3	2.2	2.2	1.8	1.2	1.5	1.1	1.4
3	17	5.5	4.5	2.5	1.7	1.7	2.5	1.5	0.9	1.2	1.5	0.8
4	22.5	8	3	2.5	3.2	2.7	1.7	2	1.1	0.8	1.4	1.9
5	20	7.5	3.5	4	2.5	2.5	1.8	1.3	1.8	1.5	1.3	1.2
6	19	6.5	5.5	3.5	3	2	2.3	0.8	1.6	1.1	1.7	0.9
7	21	5.5	4.5	4.5	2.2	1.7	1.7	1.5	1.4	1.4	1.3	1
8	20.5	7	2.5	3	2.3	2.1	2.1	1.4	1.2	1.3	0.9	1.4
9	20.5	7.5	4	1.5	1.7	1.8	1.4	1.9	1.5	1.4	1.8	1.5
10	20	8.5	3.5	2	2.6	2.3	1.5	2.2	1.8	1.7	1.2	1.3
Avg.	**20**	**7**	**4**	**3**	**2.5**	**2.2**	**1.9**	**1.6**	**1.4**	**1.35**	**1.3**	**1.3**

Graphing the Data

1. The independent (manipulated) variable in this experiment was the culture number. The independent variable is graphed on the _____ axis. Label this axis.
2. The dependent (responding variable) is the average zone of inhibition. The dependent variable is graphed on the _____axis. Label this axis.
3. Choose appropriate scales for your *x*- and *y*-axes.
4. Graph the data points.
5. The data are not continuous; therefore, you should not draw lines connecting the data points.
6. Give your graph a title.





Discussion Questions:

1. The average diameter of the zone of inhibition decreased with each new subculture. What does this imply about the bacteria in each new culture population?

_____

_____

_____

2. There was variability in the measured diameter of the zone of inhibition among the ten individual plates for each new culture.

    a. Some of this variability is due to variability in the experimental procedure (what the experimenter did). List three possible sources of experimental variability.

        i. _____

        ii. _____

        iii. _____

    b. Another explanation is related to variability among the bacteria that grew on the plate. In what way are the bacteria different? How did this happen?

_____

_____

_____

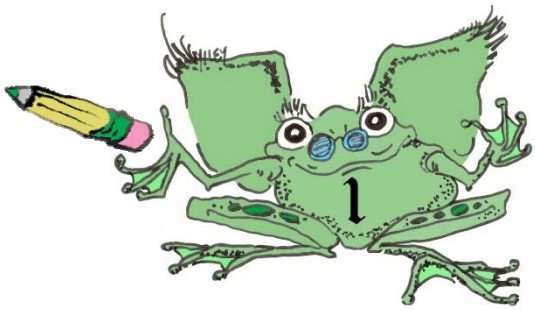
3. The average diameter of the zone of inhibition changed by a different amount with each new culture. Describe the differences between the consecutive cultures.

_____

_____

_____

4. Quantify the differences in the average diameter of the zone of inhibition between each set of consecutive cultures. In the table below, fill in the difference between the average diameter of the zone of inhibition for each culture and the culture previous to it. The first two calculations are given as examples.



Culture #	Average Diameter of the Zone of Inhibition (mm)	Difference in inhibition
1	20	
2	7	13
3	4	3
4	3	
5	2.5	
6	2.2	
7	1.9	
8	1.6	
9	1.4	
10	1.35	
11	1.3	
12	1.3	

Each of the values you calculated above represents a discrete change in the size of the zone of inhibition between two consecutive subcultures. The relationship between size of the zone of inhibition and the subculture number is not continuous or constant. The differences in $\Delta y/\Delta x$ for each two consecutive points are not due to error or biological variability, at least not totally.

5. Between which cultures is the change in the average diameter of the zone of inhibition the greatest?

   _____

6. Between which cultures is the change in the average diameter of the zone of inhibition the least?

   _____

7. What changes are occurring in the population of bacteria that would explain these differences?

   _____

   _____

   _____

8. The process of evolution is usually very slow and yet the change in the zone of inhibition is very rapid at first in this experiment. How is this example of evolution different from most other examples of evolution? (Hint: It has to do with the experimental procedure.)

   _____

   _____

   _____

9. It is not possible to write a linear equation for your graph. Why?

   _____