

## Small Group Activity 1—Cell Wall Structures and Antibiotic Action

Before class, students should have completed the provided review sheet (part I) which covers the basic components of cell envelope structure of the microorganism presented in this activity. Typically, small group instructors review the answers to this sheet at the beginning of class to refresh students' memory and also to ensure the accuracy of their answers. After the review, distribute this activity and divide the class into groups of three students. Groups are encouraged to work together to determine the correct answers to the questions, however each individual needs to write his or her own reasoning as to why the answer is correct.

You have recently been hired as an Assistant Professor where your research deals with isolating novel antibiotics to aid in the growing problem of antibiotic resistance. In your first months of work, you have isolated several interesting antimicrobial compounds which show real promise. Unfortunately, late one night you did not label the tubes clearly. Now your promising work needs to be pieced back together with only a few scraps of information scribbled on a napkin from that night. Good luck!

### Deciphered napkin scribblings...

Antibiotic A: 0.5 kDa protein, targets peptidoglycan      *983-7554 cutie from Castaways*

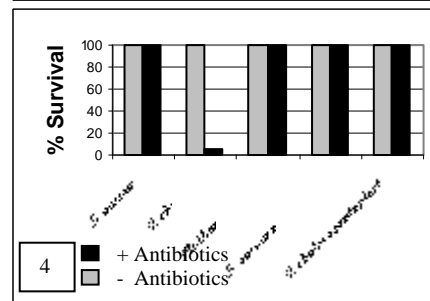
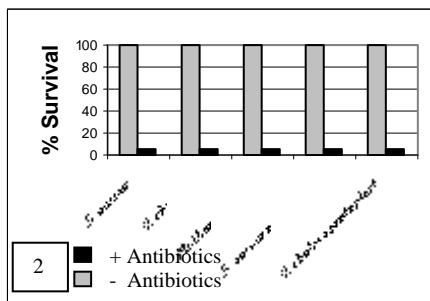
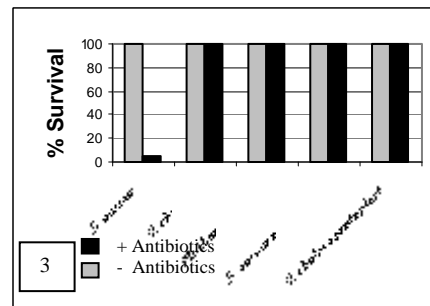
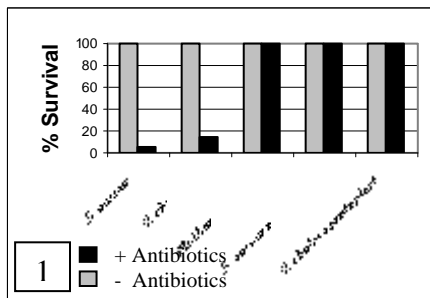
Antibiotic B: 20 kDa protein, targets peptidoglycan

Antibiotic C: Cationic antimicrobial peptide      *get!! milk, bread, cheese, stamps*

Antibiotic D: Targets lipopolysaccharide      *Bus #81 comes at 7:10*

With some more searching, you found these results in your notebook. Unfortunately they are not labeled either. These graphs represent percentage of survival of bacteria or protoplasts after treatment with the antibiotics.

Samples with antibiotic added (black bars); controls with no antibiotic added (grey bars)



### Helpful background information

*Staphylococcus aureus*: gram-positive bacterium      *Vibrio cholera*: gram-negative bacterium

*Methanosarcina*: an archaean bacterium

Cationic antimicrobial peptides (CAMPs): these positively charged antibiotics are attracted to the negatively charged cell wall and membrane. They are hydrophobic, and they insert into the membranes to create pores.

## Small Group Activity—Cell Wall Structures and Antibiotic Action Writing Assignment

Your name:

Your instructor's name:

*Complete the following questions, limiting your answers to the spaces provided*

1. Using the information on the previous page, match each antibiotic with an experiment. Fill in the blanks below and briefly discuss the results observed for each experiment, making sure to include the predicted mode of action for each antibiotic. (9 points)

Experiment #1 is Antibiotic     A    

Cell wall active antibiotic. The antibiotic in experiment #1 killed gram-positive and gram-negative bacteria and had no effect on protoplasts or archaea. This can be explained by a low molecular weight antibiotic that can pass through the porins and attack the peptidoglycan of the gram-negative bacteria and degrade the thick peptidoglycan cell wall of the gram-positive bacteria causing lysis and death of bacteria. There is no effect on archaea since it has pseudopeptidoglycan and protoplasts which have no peptidoglycan and therefore are not susceptible to the action of this antibiotic.

Experiment #2 is Antibiotic     C    

This antibiotic killed all three microorganisms as well as the protoplasts, therefore it attacks the cell membrane. This is present in the cell envelopes of all three bacteria tested and is still present in protoplasts.

Experiment #3 is Antibiotic     B    

This antibiotic killed only gram-positive cells. Therefore it is cell wall active, since it kills the gram-positive bacteria, but not the gram-positive protoplasts. It is a large antibiotic that cannot penetrate the outer membrane of the gram-negative cell (porins exclude large molecules) and therefore cannot access the peptidoglycan.

Experiment #4 is Antibiotic     D    

This antibiotic killed only gram-negative cells. Lipopolysaccharide is only found in the outer membrane of gram-negative bacteria; therefore antibiotic D only attacks the gram-negative bacteria. The protoplasts are not affected since the cell wall has been removed.

2. Pick one of the antibiotics above (A–D) and describe how a bacterial cell could become resistant to that antibiotic, i.e., what in the cell would have to change to make a bacterium resistant? (1 point)

Any of the following that seems reasonable:

1. modification of the target (different side chains or bonds in peptidoglycan, change charge of cell wall or membrane to repel cationic peptide, etc.; any ideas that are reasonable are accepted)
2. efflux of antibiotic
3. inactivation of the antibiotic: degradation or modification of antibiotic
4. altered membrane permeability