Lecture 13

I. The lethal effects of ultraviolet light

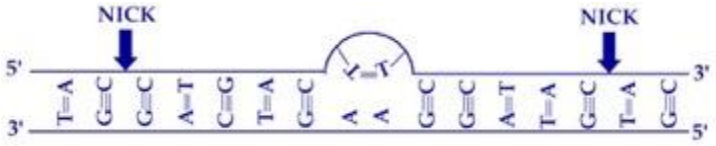
A. UV light is a short wavelength (~10-400 nm), high-energy form of radiation that harms or kills most bacteria by_____ _____.

B. When DNA is exposed to UV light, the weak hydrogen bonds between thymine (T) and adenine(A) _____ and a stronger covalent bond forms between adjacent _____ on the same strand. These are called _____ _____

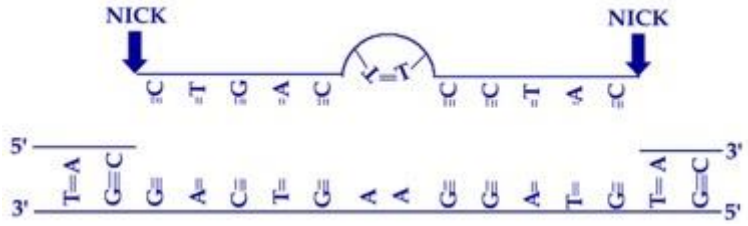
C. Thymine dimers inhibit accurate _____ _____.

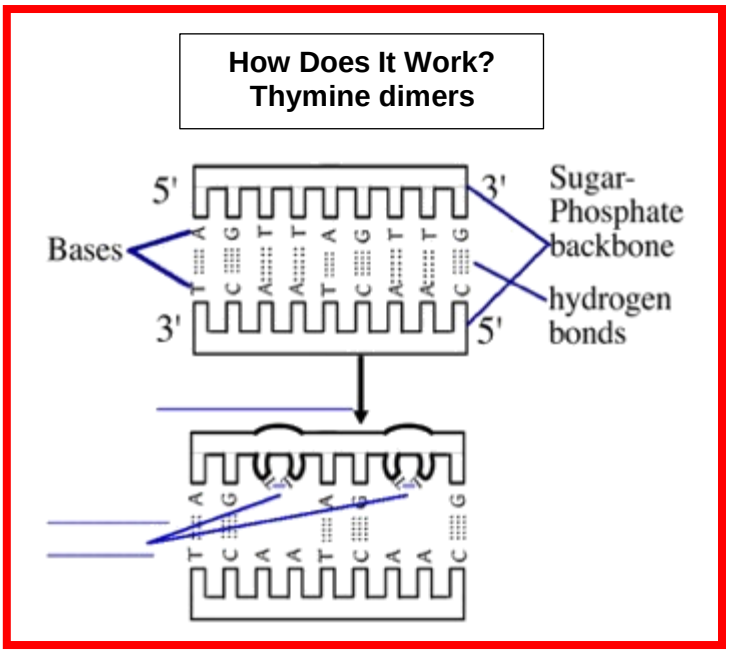
D. Accurate and complete DNA replication and transcription are _____ to the survival of bacteria. Thus, bacteria have evolved mechanisms to repair these thymine dimers.

1. _____ (light repair)
   i. The photoreactivation repair enzyme, _____, is activated by _____.
   ii. PRE uses blue light to _____ covalent bonds between thymine dimers.
   iii. After these covalent bonds are broken, the hydrogen bonds naturally reform.
2. _____
   i. An _____, called UvrABC.[1] breaks the sugar phosphate backbone of the DNA strand near the dimer on each side.

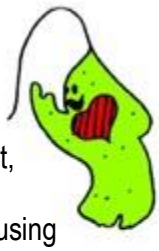


ii. The H-bonds between the base pairs are broken, and a segment of DNA ~12 nucleotides long _____.



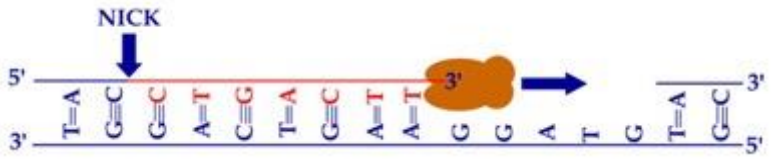


Note: If bacteria survive exposure to UV light, they are probably using one of these mechanisms of repair.



1. Cell (1983). 33 (1): 249-260

iii. DNA polymerase recognizes the 3'OH primer and _____.



iv. DNA ligase _____ in the sugar phosphate backbone.

II. Uses of ultraviolet light

A. Because of the harmful effects of UV light on bacteria, we can use it to _____ things. Despite the high energy of this form of radiation, it is not very penetrating. Thus, it can only sterilize _____ (e.g. surfaces in operating rooms, labs, and cold room lockers).

III. Antibiotics and selective toxicity

A. _____ first discovered antibiotics when he was observing a plate of *Staphylococcus aureus* that was contaminated with a *Penicillium*. He noticed that around the *Penicillium*, the growth of *S. aureus* was _____. The inhibition was caused by _____, an antibiotic produced by *Penicillium.*

B. An antibiotic is a substance that is _____.

1. Many _____ secrete antibiotics (*Actinomyces*, *Streptomyces*, and *Bacillus*)
2. Some _____, such as *Penicillium*, produce antibiotics.
3. This is often a form of biological _____ between microbes.

C. Bacteriostatic vs. bactericidal

1. Bacteriostatic agents _____ the growth and reproduction of bacteria.
2. Bactericidal agents _____ the target bacteria.
3. Some antibiotics are bacteriostatic and some are bactericidal. The effect of an agent _____ _____; it may be cidal for one species and static for another (Prescott text).

D. Selective toxicity

1. Selective toxicity is the ability of a substance to kill one _____ organism but not another.

E. Antibiotic sites of action

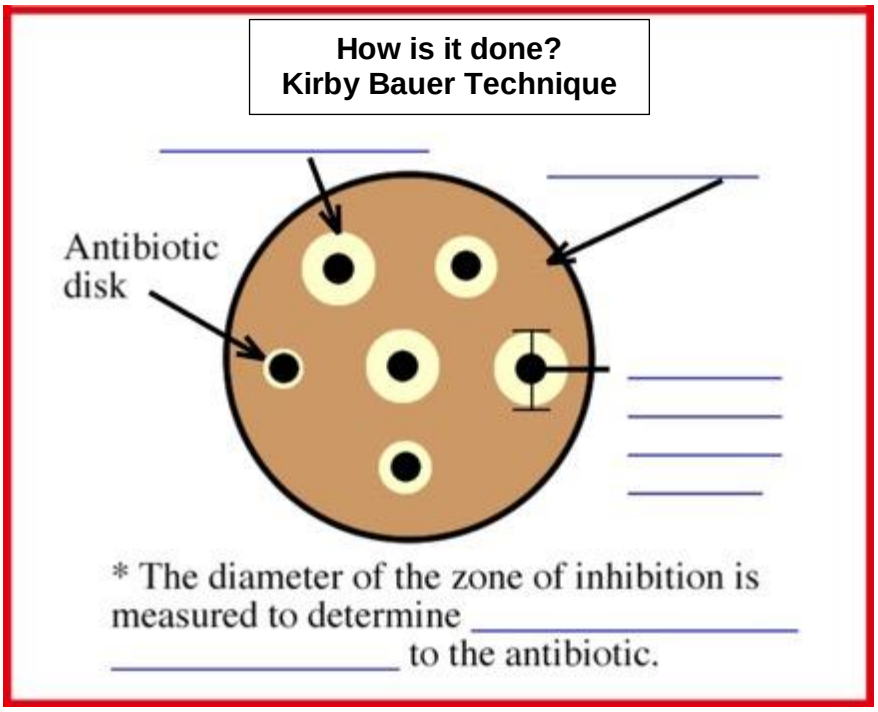
1. _____ bacteria are susceptible to all antibiotics. It is therefore important that the pathogen of interest is _____ and the _____ _____ is identified using the _____.
2. _____
   i. Works on both Gram-positive and Gram-negative bacteria
   ii. Inhibits DNA gyrases and topoisomerases. A _____ drug that's highly effective against _____.
3. Sulfa drugs:
   i. Work on Gram-positive and Gram-negative bacteria
   ii. _____ block folic acid production. These are broad-spectrum drugs, but use is limited because of antibiotic resistance and allergic reaction.
4. Penicillin
   i. Works on Gram-positive bacteria
   ii. _____. Activates cell wall lytic enzymes. Commonly used against *Staphylococci* and *Streptococci.*
5. Polymyxin
   i. Works on Gram-negative bacteria
   ii. _____. Very effective against *Pseudomonas, Escherichia*, and *Salmonella.*
6. Tetracycline, streptomycin, gentamicin, and erythromycin
   i. Considered _____
   ii. _____

IV. Kirby Bauer Technique

A. Kirby Bauer is a technique that demonstrates antimicrobial sensitivity patterns. This is used to test the _____.

B. Place small paper disks, which have been impregnated with antibiotics, onto a Mueller-Hinton agar plate that's been inoculated with the bacteria of interest.

1. As the disks absorb water, the antibiotics _____ and if effective will inhibit the growth on the plate around them.
2. The result is a lawn of bacterial growth with _____ around the discs.
3. The _____ for an antibiotic is directly proportional to the sensitivity of the isolate for that antibiotic.4. You cannot directly compare zone sizes between different antibiotic disks because they _____. Instead, they are compared to controls.



Target or mechanism	Example of an antibiotic with this target or mechanism	Often used to treat
Bacterial cell wall (peptidoglycan crosslinking)	penicillin	Gram-positive bacteria*
70S Ribosomes (Inhibit protein synthesis)	tetracycline, streptomycin, erythromycin and gentamicin	Many different bacteria (broad-spectrum drugs)
Bacterial cell membrane	nystatin and polymyxin	polymyxin-Gram-negative bacteria nystatin-fungal infections
DNA gyrase (topoisomerase) (Inhibit DNA packaging)	nalidixic acid	Many different bacteria (broad-spectrum drug)
Antimetabolite (Inhibit folic acid anabolism)	sulfa drugs (sulfonamides) (considered antibiotics even though they are synthetic agents and not microbially synthesized)	Many different bacteria (broad-spectrum drugs)*

*Because of improper use and overuse, an increasing number of bacteria are becoming resistant.

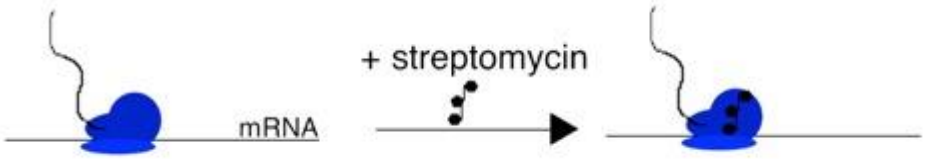
V. Microbial Genetics: Antibiotic Resistance and the Isolation of Drug-Resistant Mutants

A. A mutation is a permanent _____ in the genetic material.

1. _____: random error made by DNA polymerase during replication. Bacteria have a low rate of _____ (approximately 1 in 10 million-10 billion). Since bacteria can be grown to large numbers, we can observe these rare events.
2. Induced mutation: Physically altering the DNA with _____.
3. Although mutations often cause detrimental changes, sometimes a mutation leads to a _____ _____ (e.g. the ability to live in the presence of an antibiotic).

Resistance to streptomycin:

In a normal bacterial cell:



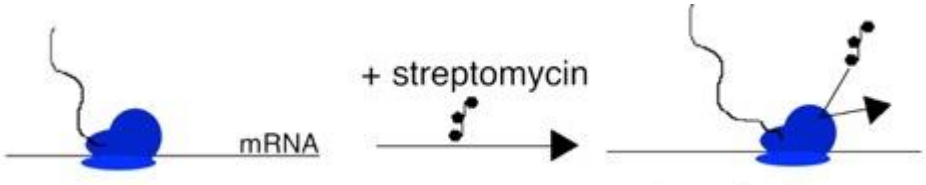
Polypeptide chain being synthesized

Streptomycin _____

_____

_____

In a mutant cell that is resistant to streptomycin



Polypeptide chain being synthesized

A mutation in the large ribosomal subunit _____

_____

_____

_____

4. How would we isolate a mutant bacterial cell with the ability to live in the presence of an antibiotic such as streptomycin?

_____

_____

B._____, but they do select for them.