NAME:_____
PLEDGE:_____

Biology 50-384 (Microbiology): Exam #3

1. You have isolated a series of mutants that have altered patterns of ß-galactosidase and lactose permease activity (i.e. they are different that the wild type pattern), which are shown in the table below. For each mutant, indicate ALL of the possible gene(s) and/or component(s) of the *lac* operon that could be mutated based on the data shown. For some mutants, there are several possibilities, all of which should be listed.

	ß-galactosidase activity		**Permease activity**	
mutant #	**- lactose**	**+ lactose**	**- lactose**	**+ lactose**
1	+	+	+	+
2	-	-	-	-
3	-	-	-	+
4	-	-/+	-	-/+

mutant #1: The mutations could have been in either lacI *(to prevent binding to the* lac *operator) or in the operator (O^c).*

mutant #2: The mutations could have been in either the lac *promoter,* lacI *(to prevent lactose from binding), or in lacZ (so to prevent entry of the inducer). Also, I accepted a mutation in* <u>both</u> lacZ *and* lacY.

mutant #3: The mutation was in lacZ.

mutant #4: The mutation could have been in Cap gene (crp), *adenylate cyclase gene (*cya*), or* lac *promoter.*

2. The following *E. coli* strains have mutations in the *trp* operon. Describe the effect of each mutation on *trpE* mRNA transcription in media with high levels of tryptophan and in media with low levels of tryptophan. Also, provide a brief (no more than 2 sentences) explanation of your answer. The *E. coli* strains all have mutations in *trpR*, so there is no transcriptional repression due to TrpR repressor.

   a) Mutations in the region 2 of the trp attenuator

   Mutations in region 2 would prevent the 2,3 antiterminator from forming. Thus the 3,4 terminator would always form and transcription of the trpE *gene would not occur, irregardless of the trp levels in the cell.*

   b) Mutations in the region 4 of the trp attenuator

   Mutations in region 4 would prevent the 3,4 terminator from forming. Thus, transcription of the trpE *gene would always occur, irregardless of the trp levels in the cell.*

   c) Mutations that change the trp codon to another codon that does not specify trp.

   Mutations that change the trp codon to one that does not specify trp would result a cell that always terminates the trp leader transcript before trpE *(not* trpE *transcription), irregardless of the trp levels. This is because the ribosome would not stall at altered codons in region 1 and instead would move down the RNA until it hit the stop codon in region 2. The ribosome would then be over region 2, preventing the 2,3 antiterminator, thus allowing the formation of the 3,4 terminator.*

3. You are studying the bacterium *Slimeus smelleus* which is motile, capsulated, auxotrophic for leucine, able to ferment lactose, sensitive to naladixic acid, able to be infected by bacteriophage T7 (lytic), and pigmented. The table below lists several mutants of *Slimeus smelleus* that you wish to isolate for future study. Fill in the following table to indicate whether you could use a genetic selection to isolate these mutant (versus a genetic screen). For those that are selectable, provide a BRIEF description of the selection.

Mutant phenotype	**Selection or screen? Pick one.**
non-motile	*screen*
non-capsulated	*screen*
prototroph for leucine	*selection*
unable to ferment lactose	*screen – but I also accepted pen enrichment*
naladixic acid resistance	*selection*
unable to be infected by phage T7	*selection*
non-pigmented	*screen*

For leucine prototroph: plate S. smelleus *on minimal media (no leucine)* → *the colonies that arise are leucine prototrophs*

For naladixic acid resistant mutants: plate S. smelleus *on media containing naladixic acid* → *the colonies that arise are naladixic acid resistant mutants*

For mutants unable to be infected by T7: mix S. smelleus *and T7 phage* → *incubate for several hours to allow phage to infect all the sensitive bacteria* → *plate the mixture on an agar plate to isolate the T7 resistant survivors*

ON THIS PAGE YOU CAN CHOOSE QUESTION #4 OR #5 TO ANSWER. IF YOU ANSWER BOTH, I WILL ONLY GRADE #4.

4. You have the isolated mutants in a certain gene that result in non-functional proteins being made. The relevant part of the sequence of the wild type and mutant proteins are shown below. For each mutant protein, suggest the most likely type of mutation (at the DNA level) responsible for the resulting mutant protein and ONE mechanism which would have resulted in that mutation type.

protein	amino acid sequence
wild type	...-Pro-Arg-Lys-Lys-Gln-His-Leu...
mutant #1	...-Pro-Arg-Lys-Lys-Pro-His-Leu...
mutant #2	...-Pro-Arg-Lys-Asn-Ser-Ilu-Asn...**

** = different from wild type throughout the rest of the protein downstream from this

mutant #1: The most likely type of mutation is a point mutation since only one amino acid was altered in the mutant. A point mutation most likely arose from a spontaneous mutation due to tautomerization of a base or spontaneous misincorporation of a base that was not repaired. I also accepted the mechanism as a basepair analogue.

mutant #2: The most likely type of mutations are either insertions or deletions, leading to a frame shift, or the insertion of a transposon, leading to an entirely different nucleotide sequence. The insertion mutation most likely arose from slippage at a direct repeat during DNA replication or from an intercalating agent. The transposition mutation would have resulted from transposition into the gene.

5. The frequency at which mutations that confer resistance to naladixic acid arise in *E. coli* is less than 1 mutant per 10^8 bacteria. In contrast, the frequency at which mutations conferring resistance to phage T4 arise in *E. coli* is 1 mutant per 10^6 bacteria. The bacterial gene encoding the phage T4 receptor on the bacterial cell surface is not essential for *E. coli* survival. Propose a reason for the lower frequency of naladixic acid resistance versus phage T4 resistance. (Hint – think about what types of mutations at the DNA level are required for each resistance).

Mutations that confer resistance to naladixic acid must be very specific (i.e. in the basepairs that code for the amino acid that naladixic acid binds but not in a basepair that is important for DNA gyrase function). Thus, the most likely mutation would be a particular point mutation. In contrast, a mutation that confers resistance to phage T4 could be any mutation that results in a nonfunctional phage T4 receptor such as point mutations that prevent the phage from binding or prevent proper folding of the protein, frameshifts anywhere in the gene, transpositions, etc. In other words, there are more possible places in the gene that the mutation can occur and thus a higher frequency.

6. You are studying an outbreak of disease caused by an ampicillin resistant bacterial pathogen (*S.t*) at a veterinary clinic. You obtain bacterial isolates from the sick animals and from environmental sources (common drinking water, hay, etc) and test their sensitivities to antibiotics. The data you obtain is shown below:

isolate #	organism	location	resistant to
1	*S.t.*	animal	ampicillin
2	*S.t.*	hay	ampicillin
3	*S.t.*	hay	none
4	*E.c.*	animal	ampicillin
5	*E.c.*	hay	ampicillin
6	*E.c.*	hay	none
7	*P.a.*	hay	ampicillin
8	*P.a.*	hay	naladixic acid

Your hypothesis is that the reason that many isolates of several different species are resistant is that the ampicillin resistance gene is being transferred among the bacteria by either transformation, transduction, or conjugation. To test this, you set up the following experiments:

EXPERIMENT 1: You mixed a culture of isolate #1 with a culture of isolate #8 for several hours → spread 100 µl of the mixture onto TSBA plus ampicillin and naladixic acid→ incubated overnight → counted hundreds of colonies.

EXPERIMENT 2: You obtained a partitioned chamber and put a culture of isolate #1 into chamber A and a culture of isolate #8 into the other chamber (B). The partition prevented anything the size of a bacterium or larger from passing through (i.e. there is no contact between bacteria on either side of the partion). → incubated several hours → spread 100 µl from chamber B onto TSBA plus ampicillin and naladixic acid → incubated overnight → counted hundreds of colonies.

EXPERIMENT 3: Same as experiment 2 but with a different partition that did not let bacteriophage pass through → obtained no colonies

EXPERIMENT 4: For the above experiments, added DNase to the liquid media with the bacteria → obtained the same number of colonies as without the DNase added.

Based on these four experiments, propose how the ampicillin resistance gene is being transferred. In your answer, you must explain what EACH experiment revealed about the mechanism of transfer. You can use the back of this sheet to answer too.

Expt 1 shows that transfer of the ampicillin resistance gene from organism to organism occurs but does not reveal the mechanism. Expt 2 shows that cell to cell contact is not needed for transfer and thus rules out conjugation. Expt 3 suggests that phage are the transfer vector because transfer does not occur when phage are prevented from crossing the membrane. Expt 4 rules out transduction because the naked DNA would have been degraded by the DNases and transfer would not have occurred if the mechanism was via transformation. Thus, transfer of ampicillin resistance in occurring by transduction.

7. How would the following items BEST be sterilized?

   empty petri plates for agar: *radiation, sterilizing gases, NOT autoclave because it would melt plastic*

   TSB agar medium: *autoclaving (not boiling because spores would still be present)*

   TSB broth: *autoclaving, filter sterilize (not boiling because spores would still be present)*

   surgical equipment: *autoclaving, sterilizing gases, radiation*

   human skin before injection: *no sterilization because you would kill human cells!!!*

8. You are investigating a newly discovered antibiotic. First, you determine the MIC (minimal inhibitory concentration) of the antibiotic for a number of bacterial species and obtain the data shown in the table below. Then, you do several Kirby-Bauer disk diffusion tests with the organisms and the antibiotic and obtain the data in the table shown below. Finally, you measure the concentration of the antibiotic in the body and find that it ranges between 10 and 20 µg/ml.

organism	MIC (µg/ml)	diameter of zone of inhibition in K-B test
#1	2.5	18
#2	5	16
#3	10	14
#4	20	12
#5	40	10
#6	80	8
#7	160	6

   Based on all this information, what is the range of the zone of inhibition in the Kirby-Bauer test for an organism if is to be considered sensitive to the antibiotic for treatment purposes? resistant? intermediate? (Hint – making a graph of the data may help)

   sensitive: *>14mm to infinity*
   intermediate: *12-14mm*
   resistant: *0 mm to <12mm*

9. Antibiotics can be grouped based on their general mechanism of action in the bacterial cell (*i.e.* inhibit protein synthesis versus inhibit DNA synthesis). Briefly describe four different classes of antibiotics based on the general functions that they affect in the cell. Give one specific example of an antibiotic and its mechanism of action for each class. Finally, propose a plausible mechanism by which an organism would be resistant to each antibiotic.

See lecture notes chapter 33

10. Why is a pathogen not always a parasite?

I accepted variety of answers here including but not limited to: A pathogen that causes disease by producing a toxin that is ingested does not have to be a parasite. A potential pathogen can be a commensal organism that gets into the wrong place in the body and then becomes a pathogen.

11. Joe Money is a businessman about to attend an important party. His stomach is bothering him, so he takes a double dose of antacid. At the party he samples a number of foods, including raw oysters. Two days after the party he experiences severe diarrhea, and is diagnosed with cholera, which is known to be caused by the bacterium *Vibrio cholerae* colonizing the small intestine and secreting cholera toxin. Samples of oysters were cultured and yielded small numbers of *V. cholerae*. How do you explain the fact that no one else at the party became ill, even though others ate the oysters?

The antacid raised the pH of the stomach allowing the V. cholerae *to pass through without being killed and colonize the small intestine. Since the other people did not take the antacid, they did not get sick because their gastric acid killed the organisms before they reached the small intestine.*
I also accepted that Joe was already ill (that is why he was feeling sick) and thus had a compromised immune system, whereas the other people had intact immune systems and could fight off the V. cholerae. *Along these lines, Joe may have had lesions in his intestine that allowed colonization.*
Although not technically correct, I also acceptable was that the antacid harmed the normal small intestinal flora, thereby allowing the V. cholerae *to colonize.*

12. Bacterial pathogens synthesize many structures and products that allow the organism to live in the niche of the human body. Describe four bacterial cellular structures or products and their roles as determinants of virulence. Each structure/product must effect a DIFFERENT determinant of virulence.

See chapter 29 lecture notes

I will email you your score for this exam as soon as it is graded. Additionally, the correct answers will be posted on the web page under Exam #3 on the lecture syllabus. If you would like your exam back, please indicate which of the following methods by checking the appropriate blank.

_____ I will pick my exam up at noon on Monday May 3rd

_____I prefer that my exam is put in campus mail on Monday May 3rd

_____I will just look at the answer key and do not need my exam back.