Have You Thanked a Microbe Today? Investigation of Beneficial Products Made Using Microbes to Enhance Comprehension of Core Microbial Concepts

Resource Type: Curriculum: Classroom

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Abstract

This inquiry-based activity encourages students to explore print and Internet resources to investigate how microbes are used to create a variety of beneficial products. The instructor introduces each product during class and students are required to provide written answers to questions relating to the product or the microbe used to make it. The questions directly correlate to material presented in lecture, but students must seek the answers outside the text and lecture materials, fostering independent research, critical thinking skills, and application of concepts presented in lecture. This activity is appropriate for use in science courses for allied health majors, biology majors who are not microbiology majors, and microbiology majors.

Activity

Invitation for User Feedback. If you have used the activity and would like to provide feedback, please send an e-mail to MicrobeLibrary@asmusa.org. Feedback can include ideas which complement the activity and new approaches for implementing the activity. Your comments will be added to the activity under a separate section labeled "Feedback." Comments may be edited.

INTRODUCTION

Learning Objectives.

Students who complete this activity will be able to:

- perform independent Internet research and evaluate the reliability of an Internet site.
- use critical-thinking skills to apply concepts learned in lecture to answer questions about beneficial products made using microbes.
- better comprehend microbiology concepts and understand their relevance in everyday applications.
- recognize the positive contributions microbes make to society.

PROCEDURE

Materials. Ideally, the instructor will actually show the product being discussed to the class, but this is not essential. All of the products are readily available for purchase, with the exception of somatostatin, Humulin, and the typhoid vaccines. Good information about each of the products is available on the Internet. (Links for each of the products are provided in the Appendix.)

Student Version.

HYTAMT? PermaGuard plant and garden insecticide
HYTAMT? Mosquito Dunks and other bioinsecticides
HYTAMT? Nitragin garden inoculant
HYTAMT? Beano and Lactaid dietary supplements
HYTAMT? Recombinant somatostatin and Humulin human proteins
HYTAMT? Neosporin first aid antibiotic ointment
HYTAMT? Vivotif and Typhim Vi typhoid vaccines

Instructor Version.

1) Each of the "Have You Thanked a Microbe Today?" (HYTAMT?) assignments consists of a series of questions that relates to a particular product made using a microbe.
Example: HYTAMT? PermaGuard plant and garden insecticide

2) The assignment for a particular product is made after I have introduced the microbiology concepts that pertain to that product in lecture. The topics addressed in the questions are listed at the beginning of the answer key for each activity.
Example: Five kingdom and three domain classification systems and cell wall structure and function, specifically, cell wall composition of diatoms.

3) At the end of the lecture period, I present the product to the students, describe its function, and when possible, show the actual product to the class.
Example: I show the students a bottle of PermaGuard plant and garden insecticide and shake out a small amount of the powder for them to see. I describe how plants, and the soil around them, are dusted with the powder to control insects that feed on the plants.

4) I then pass out the question set corresponding to the product and tell the students that the questions address microbiological concepts already presented in lecture, and that they will not find the answers in the textbook but must look for them in their lecture notes and on the Internet.

Note: When I present the first HYTAMT? assignment, I explain that it is important to evaluate Internet sites to determine if they can be trusted. I go over some guidelines for the students to use and provide them with a list of useful criteria, along with some good Internet links. (This information sheet is provided with the answer keys.)

Example: The students must look up information about the product, identify the active ingredient of microbial origin, determine what microbe is the source of this ingredient, and describe how this ingredient kills insect larvae.

5) The students are given 1 week to submit answers to the question set, and their answers are graded on accuracy and completeness. I then go over the material in class when the question sets are returned, emphasizing those microbiology concepts that were presented in lecture to demonstrate their relevance in everyday applications.

Problems and Caveats.

I have found that students will sometimes plagiarize material, particularly if they do not really understand what they are reading. To reduce the incidences of plagiarism, I include a statement in the syllabus telling students that plagiarized answers will not be graded, and I restate this policy when I make the first HYTAMT? assignment. Because it is often difficult to immediately determine if an answer is plagiarized, I have found it helpful to require students to either cite their sources or include a paper copy of the sources with their answers.

The most common complaint I receive from students is that too much time and effort are required to find the information to answer the questions. To help alleviate this problem I sometimes provide web links to help students access the information. The students must still read and understand the material in order to find the information that they need, but it reduces their sense of frustration. Another possible solution would be to have students post websites and develop a class collection of reliable web resources.

Other suggestions include weighting the assignments more heavily to better reflect the amount of effort required or allowing students to collaborate as groups on the assignments. Although I have not done this, I think that groups of students could create a poster presenting some of the products and the relevant microbiology concepts associated with them.

Suggestions for determining student learning.

Students are graded on the quality and accuracy of their written answers. I allot ten points for each HYTAMT? assignment, for a total of sixty points. These sixty points represent 12% of the total points possible in the course. A specific breakdown of point distribution follows.

Graded Exams and Assignments	**Points**
· Three in-class exams (each worth 100 points)	300
· Final exam	110
· Six HYTAMT? assignments (10 points each)	60
Total	470

Field Testing.

I have used this exercise in my General Microbiology course for the last 5 years. This is a small class of about thirty students, most of whom have a background in general biology and chemistry, but many of whom are not microbiology majors. Student evaluations reflect that these exercises helped students gain a better appreciation for the positive role microbes play in everyday life. In addition, students frequently comment that the HYTAMT? exercises helped them understand how to apply what they learned in class.

Question (84 students surveyed)	**SA**	**A**	**N**	**D**	**SD**
"The Have You Thanked a Microbe Today? assignments increased my understanding of the beneficial role microbiology plays in everyday life."	34.5%	52.4%	3.6%	3.6%	2.0%

SA = Strongly agree
A = Agree
N = Neutral
D = Disagree
SD = Strongly disagree

Student Comments

"I like being able to apply what I learn to a real life situation."
"This really made me able to apply microbiology to my life and made it something I can have and take an active role in."
"It taught me about how we can apply knowledge learned here to the real world."
"I thought these were interesting and helpful to see practical applications of our classroom knowledge."
"These were difficult, but it also helped to increase my understanding on the subject."
"I really like this. I was able to relate to the things that we talked about."
"Helpful. I didn't think I understood fermentation until the Beano assignment."
"Very useful. I liked the use of household products that you wouldn't think have anything to do with micro."

Student Data.

Students typically do very well on these assignments. The average grade received for all six of the HYTAMT? assignments is 82.38%. I have found that students who do not score as well as they would like on the written exams are especially appreciative of this opportunity to improve their grades.

SUPPLEMENTARY MATERIALS

Possible Modifications.

I have used HYTAMT? as an extra credit exercise in my large (~100 students) lecture course for nonscience majors. The majority of the students enrolled in this class have very little background in the biological sciences. Due to the large enrollment in this course, I have adapted the question sets so that they can be offered and graded electronically. Although I don't feel that students learn as much as they do when they are required to write an answer, I think they still benefit from the exercise.

Answer Keys

How to Evaluate an Internet Source
HYTAMT? PermaGuard plant and garden insecticide answer key
HYTAMT? Mosquito Dunks and other bioinsecticides answer key
HYTAMT? Nitragin garden inoculant answer key
HYTAMT? Beano and Lactaid dietary supplements answer key
HYTAMT? Recombinant somatostatin and Humulin therapeutic proteins answer key
HYTAMT? Neosporin first aid antibiotic ointment answer key
HYTAMT? Vivotif and Typhim Vi typhoid vaccines answer key

Have You Thanked a Microbe Today?
PermaGuard Garden and Plant Insecticide

Name _____

Use your lecture notes and Internet resources to answer the following questions.

A list of ingredients from the label of a bottle of PermaGuard Garden and Plant Insecticide

ACTIVE INGREDIENTS:
Pyrethrins…….. 0.1%
Technical Piperonyl Butoxide…….. 1.0%
Diatomaceous earth…….. 88.5%
INERT INGREDIENTS……10.4%
Total…… 100.0%

1. Which of the active ingredients in PermaGuard Garden and Plant Insecticide is of **microbial** origin?)

2. The microbes used to make this active ingredient are classified as:

   _____which are members of the kingdom _____and the

   domain _____**.**

3. What is the common name for the microbes that are responsible for making this ingredient? (You don't need to know the genus and species names, just the common name.)

4. Explain how or why this type of microbe is involved in the creation of this ingredient.

5. Identify the specific component in this ingredient that kills insects and its function with respect to the microbes that create it.

6. In **your own words**, explain how this component kills insects.

Have You Thanked a Microbe Today?
Mosquito Dunks and Other Bioinsecticides

Name _____

Use your lecture notes and Internet resources to answer the following questions.

A list of ingredients from the label of Mosquito Dunks, which are used to control mosquito populations.

ACTIVE INGREDIENT:
Bacillus thuringiensis Berliner var *Israelensis*, Serotype H-14 primary powder,
7000 *Aedes aegypti* international toxic units (ITU) per milligram (dry weight basis)… 10%

INERT INGREDIENTS…….. 90%

1. The bacterium *Bacillus thuringiensis* subspecies *israelensis* (Bti) is used to create a bioinsecticide for the control of mosquito larvae populations.

   a. What do members of this genus produce? (Think about a related species discussed in class.)

   b. What is the active ingredient in Bti products? (What actually kills the insect?)

   c. The bacterium produces this active ingredient at the same time that it produces _____

   d. Describe, in some detail, how Bti kills insect larvae.

   e.. Why is Bti toxic when ingested by insect larvae, but not when ingested by humans and animals?

2. Nolo Bait, which contains *Nosema locustae*, an intracellular protozoan has been licensed by the Environmental Protection Agency as a control measure for grasshoppers.

   a. How does *N. locustae* kill grasshoppers?

   b. Nolo Bait is also called "Grasshopper Spore." How is it **different from** endospores produced by bacteria?

   c. What characteristic does *N. locustae* share with the protist *Mixotricha paradoxa*, the organism discussed in the article, "The Beast With Five Genomes?"

3. *Photorhabdus luminescens* is, perhaps, the most unusual microbe yet proposed to be used as a bioinsecticide.

   a. *P. luminescens* is what type of microbe?

   b. *P. luminescens* lives in a symbiotic relationship with what other organism? Please provide a brief description of this type of organism in your answer.

   c. This symbiotic relationship would be best termed as _____.

   d. Explain the role *P. luminescens* plays in killing insect larvae.

   e. This pathogen is named for the possession of what unique characteristic?

5. As far as I know, *P. luminescens* has not yet been approved for use by the Environmental Protection Agency, possibly because a related species, *P. asymbiotica*, has been documented to cause disease in humans.

   What are the symptoms of disease caused by *P. asymbiotica*?

Have You Thanked A Microbe Today?
Nitragin Garden Inoculant

Name _____

Use your lecture notes and Internet resources to answer the following questions.

Below is the label from a package of Nitragin Garden Inoculant.

> FOR PEAS, SNAP & LIMA BEANS
>
> Contains *Rhizobium leguminosarum* biovar *viceae, Rhizobium leguminosarum* biovar *phaseoli, Bradyrhizobium* spp.
>
> Inoculate bean, pea, lima bean and sweet pea seeds with nitrogen-gathering bacteria to ensure bigger yields and better quality. These naturally occurring bacteria aid growth of plants and add to soil fertility.

1. Why is this product recommended for bean, pea, lima bean, and sweet pea plants?

2. "Nitrogen gathering" bacteria "fix" nitrogen. Write out the chemical equation for this process.

3. Is nitrogen fixation an example of an oxidation or a reduction? Explain your answer. (1)

4. Rhizobia are eubacteria. What other type of microbe fixes nitrogen? _____

   Both of these microbes are _____.

5. Molecular nitrogen (nitrogen gas) makes up 78% of the Earth's atmosphere. Explain why N_2 is unavailable for use by most organisms.

6. What do nitrogen-fixing microbes produce that allows them to use N_2? (Explain what this substance does in your answer.)

7. Nitrogen fixation must occur in an anaerobic environment or an environment with low levels of oxygen. Why?

8. *Rhizobium* spp. are aerobic bacteria. How do they provide the environment needed for nitrogen fixation?

9. a. Explain how nitrogen fixation directly aids the growth of plants. (1)

b. Many farmers practice crop rotation, planting corn or wheat in a field one year and soy beans or alfalfa in the same field the next year. What is the advantage to planting crops such as soy beans and alfalfa?

c. Where do humans and other animals acquire their nitrogen?

d. How do the nitrogen sources used by humans and other animals differ from N_2 gas?

Have You Thanked a Microbe Today?
Beano and Lactaid Dietary Supplements

Name_____

Use your lecture notes and Internet resources to answer the following questions.

The digestive supplements Beano and Lactaid are both made using microbes. Beano is an antiflatulent and Lactaid is used to reduce the cramps, gas, and diarrhea associated with lactose intolerance. Both of these products are made using microbes. Isn't microbiology glamorous?

1. *Aspergillus niger* produces the active ingredient in Beano and *Aspergillus oryzae* produces the active ingredient in Lactaid. What type of microorganisms are *A. niger* and *A. oryzae*?

2. a. What is the active ingredient in Beano?

   b. What is the function of this compound, and how does it help prevent flatulence?

3. a. What is the active ingredient in Lactaid? (0.5)

   b. What is the function of this compound? (1)

4. Both of these active ingredients are **enzymes**. Why would microbes like *A. niger* and *A. oryze* produce this type of substance? (1)

5. It is often stated that H_2S and methane gases are produced by anaerobic bacteria that ferment sugars. However, colon bacteria or coliforms, such as *Escherichia coli*, ferment sugars to acids and $C0_2$ gas. What two additional pathways might colon bacteria use to produce H_2S and methane?

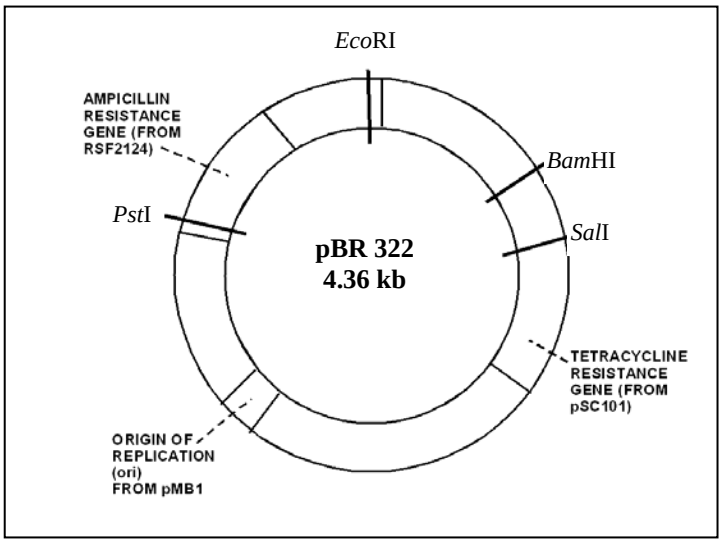
6. Explain how prokaryotes might use these two processes to produce methane and H_2S gases.

Have You Thanked a Microbe Today?
Recombinant Somatostatin and Humulin Human Proteins

Name _____

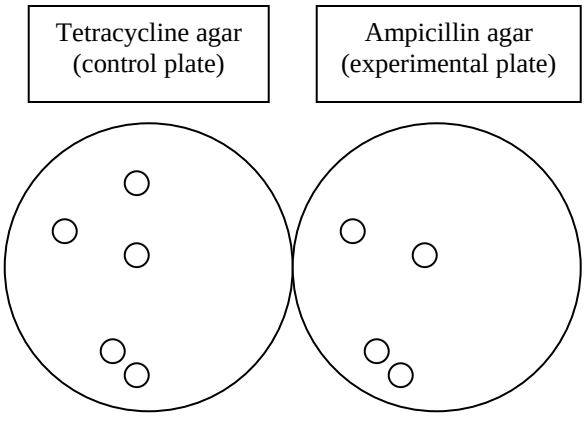
Use your lecture notes and Internet resources to answer the following questions.

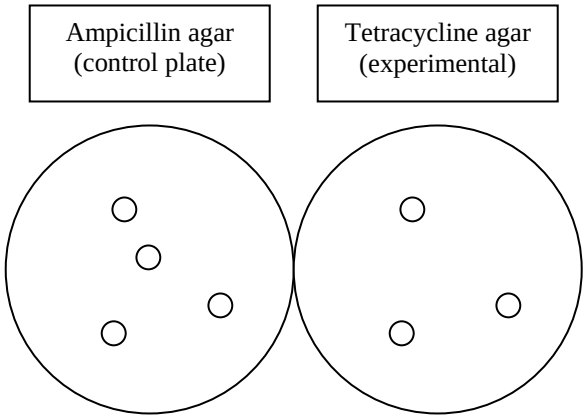
In 1977, somatostatin, a human hormone, was the first human protein to be produced in *Escherichia coli* bacteria using recombinant technology. The human gene coding for the somatostatin protein was inserted into the pBR322 *E. coli* plasmid.



The pBR322 plasmid possesses genes coding for ampicillin and tetracycline resistance. These gene markers facilitate the detection of bacteria that take up the recombinant plasmid possessing the human gene for somatostatin. The transformed bacteria are plated on agar containing one of the antibiotics. After growth, replica plating is used to test the colonies for resistance to the other antibiotic.

1. Using the diagrams below:
   a. Indicate which of the colonies is composed of bacteria containing the recombinant plasmid and explain how you know cells in the other colonies do not contain the recombinant plasmid.
   b. Identify which of the three restriction enzyme(s) (*Bam*HI, *Pst*I, *Sal*I) could have been used to produce this result.
   c. Explain how you determined which enzyme(s) could have been used.





2. Look up each of the following restriction enzymes and diagram the DNA sequence and cutting sites for each. (Hint: Look up "cutting sites" for each of the enzymes.)

   *Bam*HI
   EcoRI
   *Pst*I
   *Sal*I

3. The diagram below illustrates the DNA nucleotide sequence for the human somatostatin gene insert. Indicate on this diagram where *Eco*RI and *Bam*HI each cut the human DNA sequence. Be sure to indicate which enzyme is used to make each cut.

5' 3'
GAATTCATGGCTGGTTGTAAGAACTTCTTTTGGAAGACTTTCACTTCGTGTTAGTAGGATCC
ATTAAGTACCGACCAACATTCTTGAAGAAAACCTTCTGAAAGTGAAGCACAATCATCCTAGG

4. Recombinant somatostatin was made using *Eco*RI and *Bam*HI to remove a piece of the pBR322 plasmid. The human gene insert that was cut with these same restriction enzymes was inserted into the plasmid. The diagram below illustrates how the human somatostatin DNA and the pBR322 plasmid DNA fit together using the "sticky" ends produced by these two enzymes.

   Indicate on the diagram below which is plasmid and which is human DNA.

5' …GAATTCATGGCTGGTTGTAAGAACTTCTTTTGGAAGACTTCACTTCGTGTTAGTAGGATCC…
3' …CTTAAGTACCGACCAACATTCTTGAAGAAAACCTTCTGAAGTGAAGCACAATCATCCTAGG…

5. Using the **noncoding DNA** sequence of the somatostatin gene below:
   a. Transcribe the resulting **mRNA** sequence
   b. Identify the location of the start and stop codons for translation
   c. Use the codon table to translate the resulting amino acid sequence

G	A	A	T	T	C	A	T	G	G	C	T	G	G	T	T	G	T	A	A	G	A	A	C	T	T	C	T	T	T	T	G	G	A	A	G	A	C	T	T	T	C	A	C	T	T	C	G	T	G	T	T	G	A	T	G	A	G	A	T	C

First Position (5' End)	U	C	A	G	Third Position (3' End)
U	UUU } phe UUC } UUA } leu UUG }	UCU } UCC } UCA } ser UCG }	UAU } UAC } tyr **UAA** } **UAG** } **STOP**	UGU } UGC } cys **UGA STOP** UGG trp	U C A G
C	CUU } CUC } CUA } leu CUG }	CCU } CCC } CCA } pro CCG }	CAU } CAC } his CAA } CAG } gln	CGU } CGC } CGA } arg CGG }	U C A G
A	AUU } AUC } ile AUA } **AUG met (Start)**	ACU } ACC } ACA } thr ACG }	AAU } AAC } asn AAA } AAG } lys	AGU } AGC } ser AGA } AGG } arg	U C A G
G	GUU } GUC } GUA } val GUG }	GCU } GCC } GCA } ala GCG }	GAU } GAC } asp GAA } GAG } glu	GGU } GGC } GGA } gly GGG }	U C A G

Approval for the manufacture of recombinant human insulin occurred shortly after the introduction of recombinant somatostatin. Humulin, marketed by Eli Lily and Company in 1982, is created using a process similar to that used to make recombinant somatostatin. The following description of the product is included in their advertising.

> "Humulin is used by more than 4 million people with diabetes around the world every day. Despite its name, this insulin does not come from human beings! It is made in a factory using a chemical process called recombinant DNA technology."

Fill in the blanks to describe the step-by-step process used to make Humulin. Use each of the terms listed below in your description. Each term must be used at least once, and may be used more than once.

a) ampicillin
b) transformation
c) recombinant plasmid
d) recombinant organism
e) ligase
f) chromosome
g) human insulin
h) human insulin gene
i) restriction enzymes
j) gene vector
k) sticky ends
l) amp^r gene
m) plasmid
n) lysed

An *E. coli* cell is **6)** ____ to release a plasmid. One or more **7)** _____ are used to remove a piece of DNA from the plasmid. The same **8)** _____ are used to remove the **9)** _____ from a human **10)** _____. The cut **11)** _____ DNA and the **12)** _____ are mixed together, and complementary bonds form between the two types of DNA due to the presence of **13)** _____. **14)** _____ is added to complete annealing of the DNA segments. The resulting product, a **15)** _____, is used as a(n) **16)** _____. It is introduced into another *E. coli* cell using **17)** _____.

The **18)** _____ also contains the **19)** _____, so cells which take up the **20)** _____ can be detected because only they will grow on agar containing **21)** _____. The *E. coli* cell is a(n) **22)** _____ that will produce **23)** _____, which can be purified for use in humans.

Have You Thanked a Microbe Today?
Neosporin First Aid Antibiotic Ointment

Name _____

Use your lecture notes and Internet resources to answer the following questions.

The label below is from a tube of Neosporin.

NEOSPORIN

Polymyxin B sulfate-Bacitracin Zinc-Neomycin Sulfate

FIRST AID ANTIBIOTIC OINTMENT

Helps Prevent Infection in Minor Cuts, Scrapes, and Burns

Net Wt ½ oz (14.2 g)

1. Neosporin antibiotic ointment contains streptomycin, neomycin, and bacitracin. Of these three antibiotics, bacitracin is most similar to penicillin.

   a. What type of microbe (virus, mold, yeast, protozoan, alga) produces penicillin?

   b. What type of microbe (virus, mold, yeast, protozoan, alga) produces bacitracin?

   c. Explain how penicillin inhibits bacterial growth.

   d. Explain how bacitracin inhibits bacterial growth.

   e. Why are bacitracin and penicillin selectively more toxic to bacterial cells than human cells?

2. Penicillins and cephalosporins are beta-lactam antibiotics. Plasmids coding for the beta-lactamase enzymes make the host bacterium resistant to these antibiotics. What does beta-lactamase do?

3. Methicillin-resistant strains of *Staphylococcus aureus* (MRSA) are resistant to penicillins and often to cephalosporins. Vancomycin is often the only antibiotic that can be used to effectively treat MRSA infections.

   Vancomycin binds to the terminal D-alanine in peptidoglycan. Explain why this inhibits growth of *S. aureus.* (Please include a diagram of peptidoglycan in your answer.)

4. For some time, people have used tetracycline to treat malaria infections, although they did not know why it worked. I mentioned in class that the malarial organism possesses the remnants of a chloroplast, called an apicoplast.

   a. Antibiotics are used to treat bacterial infections. What type of microbe causes malaria?

   b. Briefly, describe how tetracycline works.

   c. Why is tetracycline more selectively toxic to bacterial cells than to human cells?

   d. The presence of the apicoplast supports what theory? Explain your answer.

   e. How does tetracycline inhibit growth of the malarial organism?

Have You Thanked a Microbe Today?
Vivotif and Typhim Vi Typhoid Vaccines

Name _____

Use your lecture notes and Internet resources to answer the following questions.

1. The information below is from a box of typhoid vaccine. Use your notes and this information to answer the following questions. (Look up live oral TY21a on the Internet for additional information.)

NDC 58337-0003-1
Typhoid Vaccine
Live Oral Ty 21a
Contains the attenuated strain
Salmonella typhi Ty21a

   a. If the label said **only** "oral Ty21a vaccine" and did **not** tell you that this is an attenuated vaccine, explain why you would still know that it is attenuated vaccine. (Hint: The little "a" does stand for attenuated, but that is not the answer I am looking for.)

   b. Explain in some detail how typhoid fever is usually contracted? (How do most people get typhoid fever?)

   c. This is a live, attenuated vaccine. Explain how the pathogen is attenuated in this vaccine and why the vaccine doesn't cause typhoid fever.

   d. This oral, attenuated typhoid vaccine stimulates both "serum antibodies" and "intestinal antibodies." What class of antibody is stimulated in the intestine? Explain your answer.

   e. People taking the Vivotif vaccine are warned against also taking sulfonamides and antibiotics. Why?

   f. Why does the dead, injected, whole-cell typhoid vaccine stimulate one type of antibody, while the oral, attenuated typhoid vaccine stimulates another? (1)

2. Another typhoid vaccine available in the U.S. is the Typhim Vi vaccine.

    a. What is the antigen in this vaccine and where does it come from? (Address cell structure in your answer.)

    b. What type of vaccine is this (attenuated, inactivated, or subunit)? Explain your answer.

Tips For Evaluating An Internet Resource

Anyone can post anything on the Internet, so it is important that you evaluate an Internet resource to determine if it can be trusted. I have listed the criteria I use to evaluate Internet sites below, as well as some useful links.

a. Authorship
   - Who wrote the article?
   - Is there contact information for this person?
   - What are this person's credentials?
   - With what organization is this person affiliated?

b. Accuracy
   - Is the information documented?
   - Are the documentation links current (not broken)?
   - Is the same information corroborated by another source?
   - Did the author use good grammar, spelling, etc.?

c. Bias
   - Who is the intended audience?
   - Does the author appear to have an "agenda?"
   - Is the information based on facts or opinion?

d. Currency
   - Is the information current? (When was the site last updated?)

Practical Steps in Evaluating Internet Resources
http://www.library.jhu.edu/researchhelp/general/evaluating/practical.html (The Sheridan Libraries, Johns Hopkins University)

Criteria for Evaluating Internet Resources
http://keithstanger.com/ineteval.htm (Keith Stanger, Eastern Michigan University Library)

Evaluating Internet Sources
http://owl.english.purdue.edu/handouts/research/r_evalsource4.html (Online Writing Lab, Purdue University)

What's a Wiki?
http://en.wikipedia.org/wiki/Wiki (Wikipedia)

ANSWER KEY
Have You Thanked a Microbe Today?
PermaGuard Garden and Plant Insecticide

Topics Covered:

- Classification—Five Kingdom and Three Domain systems
- Cell Structure and Function—Cell wall composition of diatoms

Helpful Links:

- PermaGuard product information
  - Perma-Guard Organics (http://www.permaguardorganics.com/index.html)
  - Biocontrol Network (http://www.biconet.com/crawlers/DE.html)
- Diatomaceous earth information
  - Wikipedia (http://en.wikipedia.org/wiki/Diatomaceous_earth#Pest_control)

A list of ingredients from the label of a bottle of PermaGuard Garden and Plant Insecticide

ACTIVE INGREDIENTS:
Pyrethrins…….. 0.1%
Technical Piperonyl Butoxide…….. 1.0%
Diatomaceous earth…….. 88.5%
INERT INGREDIENTS……10.4%
Total…… 100.0%

1. Which of the active ingredients in PermaGuard® Garden and Plant Insecticide is of **microbial** origin?)

   Diatomaceous earth
   (Pyrethrins are also effective against insects, but are produced by plants, not microbes.)

2. The microbes used to make this active ingredient are classified as:

   algae which are members of the kingdom ***Protista*** and the domain **Eukarya.**

3. What is the common name for the microbes that are responsible for making this ingredient? (You don't need to know the genus and species names, just the common name.)

   diatoms

4. Explain how or why this type of microbe is involved in the creation of this ingredient.

   Diatoms are found in fresh and salt water environments. When they die and settle at the bottom of the water body, their "shells" or frustules remain. Over time, these frustules accumulate and form diatomaceous earth.

5. Identify the specific component in this ingredient that kills insects and its function with respect to the microbes that create it.

   Silica. The cell walls (frustules) of diatoms are composed of silica.

6. In **your own words**, explain how this component kills insects.

   The silica is sharp and cuts the exoskeleton of the insect, causing it to dehydrate and die. Silica is also hydrophilic, absorbing water, which further desiccates the insect larvae.

ANSWER KEY
Have You Thanked a Microbe Today?
Mosquito Dunks and Other Bioinsecticides

Topics Covered:
- Cell Structure and Function—bacterial endospores
- Classification—Five Kingdom system
- Microbial Evolution—endosymbiosis
- Symbiosis—mutualism

Helpful Links:
- Mosquito Dunks label and product information
  (http://www.cliftonpark.org/ecommunity/enspec/mdunks.htm#PIC)

- *Bacillus thuringiensis* information
  American Academy of Microbiology
(http://www.asm.org/ASM/files/CCPAGECONTENT/docfilename/0000003782/Btreport%5b1%5d.pdf#search='bacillus%20thuringiensis')

- Nolo Bait product information
  Biocontrol Network
(http://www.biconet.com/biocontrol/nolo.html)
  Heirloom Garden Experts
(http://www.heirloomgardenexperts.com/pdf/nolo-bait-info-sheet.pdf#search='nolo%20bait%20label)

- *Nosema locustae* information
  MicrobeWiki, Kenyon College
(http://microbewiki.kenyon.edu/index.php/Microsporidia)

- Mystotricah paradoxa information
  "The Beast With Five Genomes," *Natural History Magazine*, June 2001
(http://nhmag.com/0601/0601_feature.html)

- *Photorhabdus luminescens* information
  Genome News Network
(http://www.genomenewsnetwork.org/articles/10_03/toxic_glow.shtml)
  University of Bath (United Kingdom)
(http://staff.bath.ac.uk/bssnw/photorhabdus_luminescens.htm)

A list of ingredients from the label of Mosquito Dunks, which are used to control mosquito populations.

ACTIVE INGREDIENT:
Bacillus thuringiensis Berliner var *Israelensis*, Serotype H-14 primary powder,
7000 *Aedes aegypti* international toxic units (ITU) per milligram (dry weight basis)… 10%

INERT INGREDIENTS…….. 90%

1. The bacterium *Bacillus thuringiensis* subspecies *israelensis* (Bti) is used to create a bioinsecticide for the control of mosquito larvae populations.

   a. What do members of this genus produce? (Think about a related species discussed in class.)

      Endospores

   b. What is the *active ingredient* in Bti products? (What actually kills the insect?)

      parasporal crystals composed of a protein endotoxin (delta toxin)

c. The bacterium produces this active ingredient at the same time that it produces **endospores**

d. Describe, in some detail, how Bti kills insect larvae.

The insect larva ingests the protein endotoxin. The endotoxin is activated in the larva's gut to a toxic form. The toxin essentially paralyzes the gut, causing the insect to starve and die.

In products that also contain Bt spores, the spores will germinate into bacteria, which will spread throughout the larva (septicemia), which will also kill the insect.

e. Why is Bti toxic when ingested by insect larvae but not when ingested by humans and animals?

The protein is only active in an alkaline pH. Humans and animals have an acidic pH (pH 2 to 3) in the stomach, which inactivates the protein, while the midguts of many insect larvae have an alkaline pH (pH 8 to 10.5), which allows proteases to cleave the protein into a toxic form.

2. Nolo Bait, which contains *Nosema locustae* an intracellular protozoan, has been licensed by the Environmental Protection Agency as a control measure for grasshoppers.

a. How does *N. locustae* kill grasshoppers?

The organism produces reproductive spores. As these spores germinate into progeny protists, they produce a filament that pierces the walls of the cells in the grasshopper gut. The protists then spread throughout the body of the grasshopper (septicemia) resulting in lethargy, reduced growth rates, and death.

b. Nolo Bait is also called "Grasshopper Spore." How is it **different from** endospores produced by bacteria?

Grasshopper spore is a reproductive mechanism for the protozoan, whereas bacterial endospores are a means of survival in hostile environmental conditions.

c. What characteristic does *N. locustae* share with the protist *Mixotricha paradoxa*, the organism discussed in the article, "The Beast With Five Genomes?"

Neither possess mitochondria. *M. paradoxa* possesses bacterial symbionts that produce ATP. It is not yet known how *N. locustae* produces ATP.

3. *Photorhabdus luminescens* is, perhaps, the most unusual microbe yet proposed to be used as a bioinsecticide.

a. *P. luminescens* is what type of microbe?

bacterium

b. *P. luminescens* lives in a symbiotic relationship with what other organism? Please provide a brief description of this type of organism in your answer.

***P. luminescens* are gut symbionts of *Heterorhabditis bacteriophora*, a nematode. (Nematodes are a type of worm and are therefore animals.)**

c. This symbiotic relationship would be best termed as **mutualistic.**

d. Explain the role *P. luminescens* plays in killing insect larvae.

When the nematode burrows into an insect larva, the bacteria release toxins that kill the insect but not the nematode host. Both the nematode and the bacteria benefit by consuming the dead insect.

e. This pathogen is named for the possession of what unique characteristic?

bioluminescence (It glows!)

5. As far as I know, *P. luminescens* has not yet been approved for use by the Environmental Protection Agency, possibly because a related species, *P. asymbiotica*, has been documented to cause disease in humans.

What are the symptoms of disease caused by *P. asymbiotica*?

Pustular sores on the skin, which sometimes result in septic disease.

ANSWER KEY
Have You Thanked A Microbe Today?
Nitragin Garden Inoculant

Topics Covered:

- Cell Energy Metabolism—metabolic pathways (oxidation and reduction; organic and inorganic compounds)
- Symbiosis—mutualism
- Microbial Recycling of Resources—nitrogen cycle; nitrogen fixation

Helpful Links:

- Garden Inoculant Product Information and Sources
  Seeds of Change Certified Organic (Nitragin Garden Inoculant)
(http://www.seedsofchange.com/garden_center/product_details.asp?item_no=S16450&UID)
  Territorial Seed Company (Nature's Aid Inoculant)
(http://www.territorial-seed.com/stores/1/Garden_Combo_Inoculant_P4261.cfm)
- Legumes
  International Legume Database & Information Service – (Family Leguminosae or *Fabaceae*)
(http://www.ildis.org/Leguminosae/)
- Nitrogen Fixation and the Nitrogen Cycle
  Institute of Cell and Molecular Biology, The University of Edinburgh
(http://helios.bto.ed.ac.uk/bto/microbes/nitrogen.htm)

Below is the label from a package of Nitragin Garden Inoculant.

> FOR PEAS, SNAP & LIMA BEANS
>
> Contains *Rhizobium leguminosarum* biovar *viceae*, *Rhizobium leguminosarum* biovar *phaseoli*, *Bradyrhizobium* spp.
>
> Inoculate bean, pea, lima bean and sweet pea seeds with nitrogen-gathering bacteria to ensure bigger yields and better quality. These naturally occurring bacteria aid growth of plants and add to soil fertility.

1. Why is this product recommended for bean, pea, lima bean, and sweet pea plants?

   Beans, peas, and lima beans are all legumes (belonging to the family *Fabaceae*). Legumes are plants that harbor nitrogen-fixing bacteria in their roots.

2. "Nitrogen gathering" bacteria "fix" nitrogen. Write out the chemical equation for this process.

   $N_2 + 8H+ + 8e^- + 16\ ATP = 2NH_3 + H_2 + 16ADP + 16\ Pi$

3. Is nitrogen fixation an example of an oxidation or a reduction? Explain your answer. (1)

   This is an example of a reduction, because N_2 gas accepts hydrogen atoms to form NH_3 (ammonia).

4. Rhizobia are eubacteria. What other type of microbe fixes nitrogen? **cyanobacteria**
   Both of these microbes are **prokaryotes**.

5. Molecular nitrogen (nitrogen gas) makes up 78% of the Earth's atmosphere. Explain why N_2 is unavailable for use by most organisms.

Nitrogen gas consists of two nitrogen atoms tightly bound to one another by three covalent bonds. Most organisms are unable to cleave these bonds.

6. What do nitrogen-fixing microbes produce that allows them to use N_2? (Explain what this substance does in your answer.)

Nitrogen-fixing microbes are the only organisms on Earth that produce the enzyme nitrogenase. Nitrogenase catalyzes the reaction in which the strong triple bonds holding the two nitrogen atoms together are broken.

7. Nitrogen fixation must occur in an anaerobic environment or an environment with low levels of oxygen. Why?

The nitrogenase enzyme is composed of two iron-containing proteins. Oxygen readily binds to the iron components of the enzyme, inactivating it.

8. *Rhizobium* spp. are aerobic bacteria. How do they provide the environment needed for nitrogen fixation?

Legumes infected with rhizobia produce leghemoglobin, a compound that reduces the amount of oxygen in the cytoplasm of infected plant cells. (Legumes that are not infected with rhizobia do not produce leghemoglobin.)

9. a. Explain how nitrogen fixation directly aids the growth of plants. (1)

Many plants are able to use ammonia (NH_3), the end product of nitrogen fixation, as a source of nitrogen.

b. Many farmers practice crop rotation, planting corn or wheat in a field one year and soy beans or alfalfa in the same field the next year. What is the advantage to planting crops such as soy beans and alfalfa?

Planting crops such as soy beans and alfalfa eliminates or greatly reduces the need for commercial nitrogen fertilizer.

c. Where do humans and other animals acquire their nitrogen?

From the animal and plant proteins that we eat

d. How do the nitrogen sources used by humans and other animals differ from N_2 gas?

Nitrogen gas is an inorganic compound. Humans and other animals require an organic nitrogen source.

ANSWER KEY
Have You Thanked a Microbe Today?
Beano and Lactaid Dietary Supplements

Topics Covered:

- Classification—Five Kingdom and Three Domain systems
- Cell Energy Metabolism—metabolic pathways (aerobic and anaerobic respiration, fermentation, methanogenesis, nutritional types, oxidation and reduction reactions)

Helpful Links:

- Beano Product information (http://www.beanogas.com/)
- Lactaid Product information (http://www.lactaid.com/)
- Carbohydrate Intolerance Information
  Complex Carbohydrate Intolerance Information Center - Alpha-Galactosidase (http://www.preventcci.com/professionals/default.aspx)
  Great Vista Chemicals -Lactase (Beta-Galactosidase) (http://www.greatvistachemicals.com/biochemicals/lactase.html)
  The Skinny on Why Beans Give You Gas, *Discovery*.com (http://www.discovery.com/area/skinnyon/skinnyon970815/skinnyon.html)
- Metabolic Pathways
  Central Michigan University (prokaryotic metabolic pathways) (http://www.cst.cmich.edu/users/alm1ew/208%20Metabolism%20concepts.htm)
  University of Wisconsin-Madison (methanogens) (http://www.bact.wisc.edu/Microtextbook/index.php?name=Sections&req=viewarticle&artid=94&allpages=1&theme=Printer)
  Answers.com (anaerobic respiration) (http://www.answers.com/topic/hydrogen-sulfide)

The digestive supplements Beano and Lactaid are both made using microbes. Beano is an antiflatulent and Lactaid is used to reduce the cramps, gas, and diarrhea associated with lactose intolerance. Both of these products are made using microbes. Isn't microbiology glamorous?

1. *Aspergillus niger* produces the active ingredient in Beano and *Aspergillus oryzae* produces the active ingredient in Lactaid. What type of microorganisms are *A. niger* and *A. oryzae*?

   They are both molds.

2. a. What is the active ingredient in Beano?

      alpha-galactosidase

   b. What is the function of this compound, and how does it help prevent flatulence?

      Alpha-galactosidase breaks large, complex carbohydrates called oligosaccharides into smaller, simpler sugars. Oligosaccharides are composed of monosaccharides linked to one another by alpha-galactosidic linkages. Humans don't produce alpha-galactosidase, so they are unable to break these large sugars into the simple sugars that can be absorbed in the small intestine. Bacteria in the large intestine that do produce alpha-galactosidase can break down the large sugars, producing gas in the process.

3. a. What is the active ingredient in Lactaid? (0.5)

      lactase (beta-galactosidase)

   b. What is the function of this compound? (1)

Beta-galactosidase breaks the disaccharide lactose into two monosaccharides, galactose and glucose. Many people do not produce sufficient amounts of lactase to efficiently digest lactose in the small intestine. Lactase-producing bacteria in the large intestine break down the lactose, producing gas in the process.

4. Both of these active ingredients are **enzymes**. Why would microbes like *A. niger* and *A. oryze* produce this type of substance? (1)

Molds, like other fungi, absorb their nutrients. In order to do this, they secrete enzymes that break down large substrates into smaller units that can be absorbed.

5. It is often stated that H_2S and methane gases are produced by anaerobic bacteria that ferment sugars. However, colon bacteria or coliforms, such as *Escherichia. coli*, ferment sugars to acids and $C0_2$ gas. What two additional pathways might colon bacteria use to produce H_2S and methane?

Anaerobic respiration
Autotrophic methanogenesis

6. Explain how prokaryotes might use these two processes to produce methane and H_2S gases.

Anaerobic respiration:

- **An organic sulfur-containing compound serves as the carbon, energy, and electron source.**
- **Sulfur or sulfate serves as the electron acceptor, forming hydrogen sulfide gas. ($SO_4^{2-} + 10H^+ + 8^{e-} \rightarrow H_2S + 4H_2O$)**
- **Acetate serves as the electron acceptor, forming methane (acetotrophic methanogenesis). ($CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$)**

Autotrophic methanogenesis:

- **Carbon dioxide serves as the carbon source and electron acceptor. Hydrogen gas reduces the carbon dioxide, forming methane. ($CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$)**

ANSWER KEY
Have You Thanked a Microbe Today?
Recombinant Somatostatin and Humulin Human Proteins

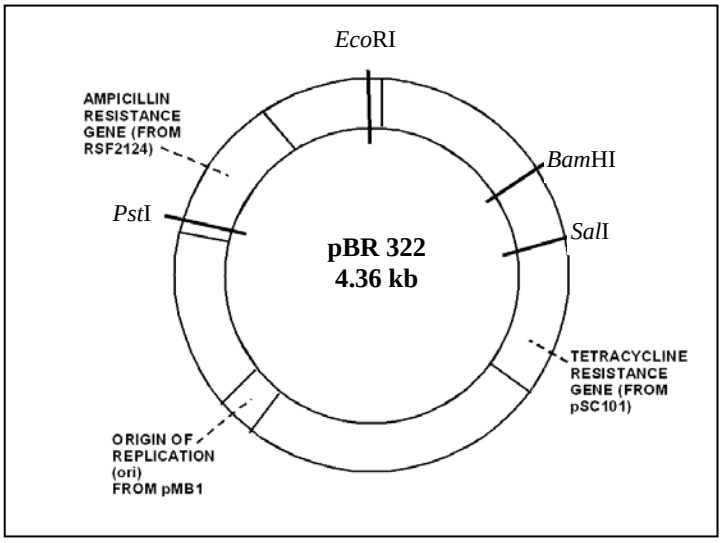
Topics Covered:

- Microbial Genetics—Inheritance of genetic information
- Interaction and Impact of Microorganisms and Humans—Genetic engineering and biotechnology

Helpful Links:

- Somatostatin Information
  - UCB (http://www.ucb-group.com/products/allergy_respiratory_PC/somatostatin-ucb/)
  - University of Westminster, UK (http://www.wmin.ac.uk/~redwayk/lectures/vectors.htm)
  - Fermentas (http://www.fermentas.com/techinfo/nucleicacids/mappbr322.htm)
- pBR322 DNA sequence
  - Cardiff University, UK (http://www.cf.ac.uk/biosi/staff/ehrmann/tools/dna/pBR322.html)
- Restriction Enzymes
  - Access Excellence (definition) (http://www.accessexcellence.org/AE/AEC/CC/restriction.html)
  - Access Excellence (chart of examples) (http://www.accessexcellence.org/AE/AEC/CC/re_chart.html)
  - *Sal*I Cutting Sites (RecPro Biotech) (http://www.rpbiotech.com/Restriction_enzymes/SalI.htm)
  - *Pst*I Cutting Sites (RecPro Biotech) (http://www.rpbiotech.com/Restriction_enzymes/pstI.htm)
  - *Bam*HI Cutting Sites (RecPro Biotech) (http://www.rpbiotech.com/Restriction_enzymes/BamHI.htm)

In 1977, somatostatin, a human hormone, was the first human protein to be produced in *Escherichia coli* bacteria using recombinant technology. The human gene coding for the somatostatin protein was inserted into the pBR322 *E. coli* plasmid.



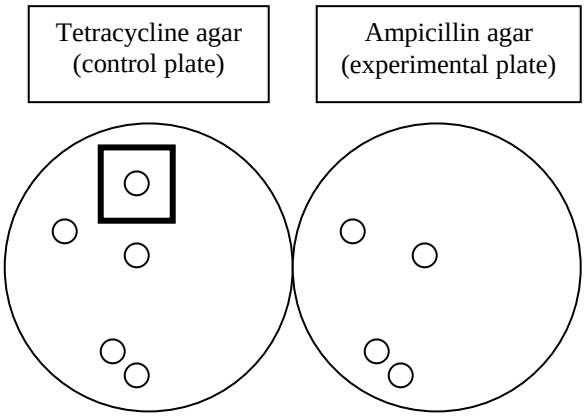
The pBR322 plasmid possesses genes coding for ampicillin and tetracycline resistance. These gene markers facilitate the detection of bacteria that take up the recombinant plasmid possessing the human gene for somatostatin. The transformed bacteria are plated on agar containing one of the antibiotics. After growth, replica plating is used to test the colonies for resistance to the other antibiotic.

1. Using the diagrams below:
   a. Indicate which of the colonies is composed of bacteria containing the recombinant plasmid and explain how you know cells in the other colonies do not contain the recombinant plasmid.
   b. Identify which of the three restriction enzyme(s) (*Bam*HI, *Pst*I, *Sal*I) could have been used to produce this result.
   c. Explain how you determined which enzyme(s) could be have been used.

a. The boxed colony represents a transformed colony of *E. coli* cells. Cells that were not transformed with the recombinant plasmid are able to grow in the presence of either antibiotic.

b. **The restriction enzyme *Pst*I would cut the ampicillin gene in the plasmid, rendering it inactive.**

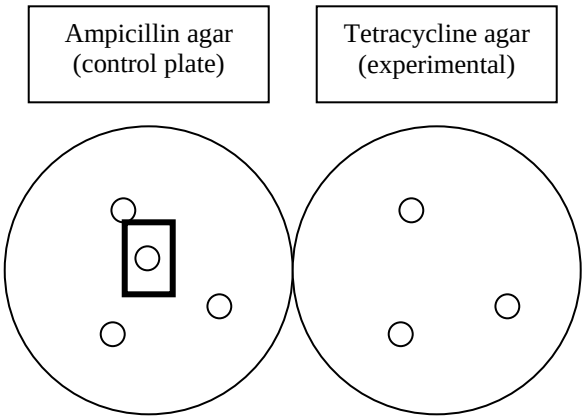
c. **Therefore, cells that were transformed with the recombinant plasmid would be able to grow in the presence of tetracycline, but not in the presence of ampicillin.**



a. The boxed colony represents a transformed colony of *E. coli* cells. Cells that were not transformed with the recombinant plasmid are able to grow in the presence of either antibiotic.

b. Either of the restriction enzymes *Bam*HI and *Sal*1 would cut the tetracycline gene in the plasmid, rendering it inactive.

c. Therefore, cells that were transformed with the recombinant plasmid would be able to grow in the presence of ampicillin, but not in the presence of tetracycline.



2. Look up each of the following restriction enzymes and diagram the DNA sequence and cutting sites for each. (Hint: Look up "cutting sites" for each of the enzymes.)

*Bam*HI
EcoRI
*Pst*I
*Sal*I

***Bam*HI** **G – G – A – T – C – C**
C – C – T – A – G – G

***Eco*RI** **G – A – A – T – T – C**
C – T – T – A – A – G

***Pst*I** **C – T - G – C – A – G**
G – A – C – G – T – C

***Sal*I** **G – T – C – G – A – C**
C – A – G – C – T – G

3. The diagram below illustrates the DNA nucleotide sequence for the human somatostatin gene insert. Indicate on this diagram where *Eco*RI and *Bam*HI each cut the human DNA sequence. Be sure to indicate which enzyme is used to make each cut.

5' 3'

***Eco*RI** ***Bam*HI**

G AATTCATGGCTGGTTGTAAGAACTTCTTTTGGAAGACTTTCACTTCGTGTTAGTAG GATCC
ATTAA GTACCGACCAACATTCTTGAAGAAAACCTTCTGAAAGTGAAGCACAATCATCCTAG G

4. Recombinant somatostatin was made using *Eco*RI and *Bam*HI to remove a piece of the pBR322 plasmid. The human gene insert that was cut with these same restriction enzymes was inserted into the plasmid. The diagram below illustrates how the human somatostatin DNA and the pBR322 plasmid DNA fit together using the "sticky" ends produced by these two enzymes.

Indicate on the diagram below which is plasmid and which is human DNA.

5' …G**AATTCATGGCTGGTTGTAAGAACTTCTTTTGGAAGACTTCACTTCGTGTTAGTAG**GATCC…
3' …CTTAA**GTACCGACCAACATTCTTGAAGAAAACCTTCTGAAGTGAAGCACAATCATCCTAG**G..

- Human somatostatin gene **in bold**
- Plasmid DNA in not in bold

5. Using the **noncoding DNA** sequence of the somatostatin gene below:
   a. Transcribe the resulting **mRNA** sequence
   b. Identify the location of the start and stop codons for translation
   c. Use the codon table to translate the resulting amino acid sequence

G	A	A	T	T	C	A	T	G	G	C	T	G	G	T	T	G	T	A	A	G	A	A	C	T	T	C	T	T	T	T	G	G	A	A	G	A	C	T	T	T	C	A	C	T	T	C	G	T	G	T	T	G	A	T	G	A	G	A	T	C
G	A	A	U	U	C	**A**	**U**	**G**	G	C	U	G	G	U	U	G	U	A	A	G	A	A	C	U	U	C	U	U	U	U	G	G	A	A	G	A	C	U	U	U	C	A	C	U	U	C	G	U	G	U	U	G	A	U	G	A	G	A	U	C
						Met			Ala			Gly			Cys			Lys			Asn			Phe			Phe			Trp			Lys			Thr			Phe			Thr			Ser			Cys																	

Aug start codon

UGA stop codons

First Position (5' End)

	U	**C**	**A**	**G**	
U	UUU, UUC } phe UUA, UUG } leu	UCU, UCC, UCA, UCG } ser	UAU, UAC } tyr **UAA, UAG } STOP**	UGU, UGC } cys **UGA STOP** UGG trp	U C A G
C	CUU, CUC, CUA, CUG } leu	CCU, CCC, CCA, CCG } pro	CAU, CAC } his CAA, CAG } gln	CGU, CGC, CGA, CGG } arg	U C A G
A	AUU, AUC, AUA } ile **AUG met (Start)**	ACU, ACC, ACA, ACG } thr	AAU, AAC } asn AAA, AAG } lys	AGU, AGC } ser AGA, AGG } arg	U C A G
G	GUU, GUC, GUA, GUG } val	GCU, GCC, GCA, GCG } ala	GAU, GAC } asp GAA, GAG } glu	GGU, GGC, GGA, GGG } gly	U C A G

Third Position (3' End)

Approval for the manufacture of recombinant human insulin occurred shortly after the introduction of recombinant somatostatin. Humulin, marketed by Eli Lily and Company in 1982, is created using a process similar to that used to make recombinant somatostatin. The following description of the product is included in their advertising.

> "Humulin is used by more than 4 million people with diabetes around the world every day. Despite its name, this insulin does not come from human beings! It is made in a factory using a chemical process called recombinant DNA technology."

Fill in the blanks to describe the process used to make Humulin step-by-step. Use each of the terms listed below in your description. Each term must be used at least once, and may be used more than once.

a) ampicillin
b) transformation
c) recombinant plasmid
d) recombinant organism
e) ligase
f) chromosome
g) human insulin
h) human insulin gene
i) restriction enzymes
j) gene vector
k) sticky ends
l) amp^r gene
m) plasmid
n) lysed

An *E. coli* cell is **6) lysed** to release a plasmid. One or more **7) restriction enzymes** are used to remove a piece of DNA from the plasmid. The same **8) restriction enzymes** are used to remove the **9) human insulin gene** from a human **10) chromosome**. The cut **11) plasmid** DNA and the **12) human insulin gene** are mixed together, and complementary bonds form between the two types of DNA due to the presence of **13) sticky ends**. **14) Ligase** is added to complete annealing of the DNA segments. The resulting product, a **15) recombinant plasmid**, is used as a(n) **16) gene vector**. It is introduced into another *E. coli* cell using **17) transformation**. The **18) recombinant plasmid** also contains the **19) amp^r gene**, so cells which take up the **20) recombinant plasmid** can be detected because only they will grow on agar containing **21) ampicillin.** The *E. coli* cell is a(n) **22) recombinant organism** that will produce **23) human insulin,** which can be purified for use in humans.

ANSWER KEY
Have You Thanked a Microbe Today?
Neosporin First Aid Antibiotic Ointment

Topics Covered:

- Classification—Five Kingdom system
- Cellular Structure and Function—cell wall (peptidoglycan) structure
- Microbial Evolution—endosymbiosis
- Antibiotics and Chemotherapy—Mechanisms of antibiotic action; selective toxicity

Helpful Links:

- Neosporin product information (http://www.pfizerch.com/product.aspx?id=364)
- Antibiotics—Microbial Sources and Mechanisms of Action
  Chopra I, Hawkey PM, Hinton M. 1992. Tetracyclines, molecular and clinical aspects. J. Antimicrob. Chemother. **29:** 245-277.
  Elmhurst College Virtual Chembook
  (http://www.elmhurst.edu/~chm/vchembook/654antibiotic.html)
  University of Texas Medical Branch (UTMB) (http://gsbs.utmb.edu/microbook/ch011.htm)
- Beta-lactamase
  Methylgene–explanation and animation
  (http://www.methylgene.com/images/gestion/BetaLactamase.swf)
- Endosymbiosis
  Understanding Evolution by Lynn Margulis
  (http://evolution.berkeley.edu/evolibrary/article/0_0_0/history_24)
  Sumanas, Inc.
  (http://www.sumanasinc.com/webcontent/anisamples/nonmajorsbiology/organelles.html)
- Malaria apicoplast
  Nature.com
  (http://origin.www.nature.com/nature/journal/v419/n6906/full/nature01097.html)
  Kimball's Biology Pages
  (http://home.comcast.net/~john.kimball1/BiologyPages/E/Endosymbiosis.html#apicoplast)

The label below is from a tube of Neosporin.

NEOSPORIN

Polymyxin B sulfate-Bacitracin Zinc-Neomycin Sulfate

FIRST AID ANTIBIOTIC OINTMENT

Helps Prevent Infection in Minor Cuts, Scrapes, and Burns

Net Wt ½ oz (14.2 g)

1. Neosporin antibiotic ointment contains streptomycin, neomycin, and bacitracin. Of these three antibiotics, bacitracin is most similar to penicillin.

   a. What type of microbe (virus, mold, yeast, protozoan, alga) produces penicillin?

   ***Penicillium* spp. molds**

   b. What type of microbe (virus, mold, yeast, protozoan, alga) produces bacitracin?

***Bacillus* spp. bacteria**

c. Explain how penicillin inhibits bacterial growth.

Penicillin inhibits the formation of cross-linkages between the amino acid side chains in peptidoglycan, thereby preventing cell wall synthesis.

d. Explain how bacitracin inhibits bacterial growth.

Bacitracin also inhibits peptidoglycan synthesis by interfering with the lipid carrier molecule responsible for transporting peptidoglycan precursors across the bacterial cell membrane.

e. Why are bacitracin and penicillin selectively more toxic to bacterial cells than human cells?

Humans cells have no cell wall, so there is no need for peptidoglycan synthesis.

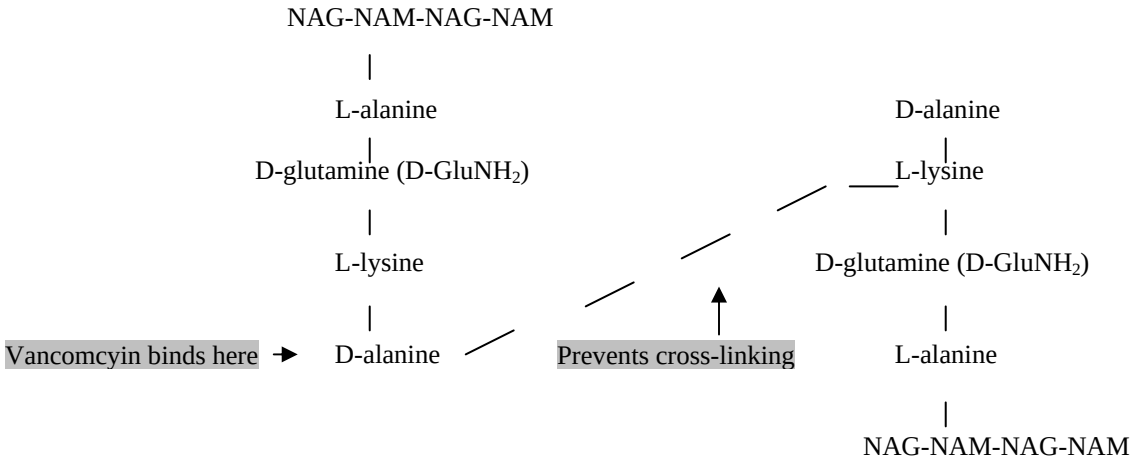
2. Penicillins and cephalosporins are beta-lactam antibiotics. Plasmids coding for the beta-lactamase enzymes make the host bacterium resistant to these antibiotics. What does beta-lactamase do?

Beta-lactamase cleaves the beta-lactam ring, rendering the antibiotics nonfunctional.

3. Methicillin-resistant strains of *Staphylococcus aureus* (MRSA) are resistant to penicillins and often to cephalosporins. Vancomycin is often the only antibiotic that can be used to effectively treat MRSA infections.

Vancomycin binds to the terminal D-alanine in peptidoglycan. Explain why this inhibits growth of *S. aureus*. (Please include a diagram of peptidoglycan in your answer.)

When vancomycin binds to the terminal D-alanine in the amino acid side chain, it prevents formation of the peptide linkage with the L-lysine group on an adjacent amino acid side chain.



4. For some time, people have used tetracycline to treat malaria infections, although they did not know why it worked. I mentioned in class that the malarial organism possesses the remnants of a chloroplast, called an apicoplast.

a. Antibiotics are used to treat bacterial infections. What type of microbe causes malaria?

protozoan

b. Briefly, describe how tetracycline works.

Tetracycline inhibits protein synthesis because it binds to the small 30S ribosomal unit and prevents aminoacyl tRNA from binding to the A site, which stops chain elongation.

c. Why is tetracycline more selectively toxic to bacterial cells than to human cells?

Human cells don't take up tetracycline as efficiently as bacterial cells, and the human cytoplasmic ribosomes are not affected at the concentration that does accumulate. (Interestingly, human mitochondrial ribosomes are affected, which further supports the endosymbiotic theory that mitochondria were once bacterial symbionts!)

d. The presence of the apicoplast supports what theory? Explain your answer.

Endosymbiosis. The apicoplast appears to be the remnant of a chloroplast. According to the endosymbiotic theory, chloroplasts are themselves remnants of photosynthetic bacteria that were taken up by a primitive prokaryote and became symbionts.

e. How does tetracycline inhibit growth of the malarial organism?

Tetracycline targets the 30S ribosomal unit in the protozoan apicoplast, as well as the mitochonodrial ribosomes of the protozoan. The apicoplast proteins are required for survival of the malarial protist, and disruption of the apicoplast, in addition to its effect on the mitochondria, kills the protozoan.

ANSWER KEY
Have You Thanked a Microbe Today?
Vivotif and Typhim Vi Typhoid Vaccines

Topics Covered:

- Disease Transmission—typhoid fever
- Cell Structure and Function and Microbial Pathogenicity Mechanisms—lipopolysaccharide
- Interaction and impact of microorganisms and humans
  - Vaccines—types and mechanism of action
  - Specific immunity

Helpful Links:

- Typhoid Vaccine Information
  Drug Information Online (http://www.drugs.com/cdi/typhim_vi.html)
  World Health Organization (WHO)
(http://www.who.int/immunization/topics/typhoid/en/index.html)
  RxMed (http://www.rxmed.com). Search for Typhum vi in the list of drugs.
  CDC MMWR (http://www.cdc.gov/mmwr/PDF/rr/rr4314.pdf)

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1. The information below is from a box of typhoid vaccine. Use your notes and this information to answer the following questions. (Look up live oral TY21a on the Internet for additional information.)

NDC 58337-0003-1
Typhoid Vaccine
Live Oral Ty 21a
Contains the attenuated strain
Salmonella typhi Ty21a

a. If the label said **only** "oral Ty21a vaccine" and did **not** tell you that this is an attenuated vaccine, explain why you would still know that it is attenuated vaccine. (Hint: The little "a" does stand for attenuated, but that is not the answer I am looking for.)

Only certain attenuated vaccines can be administered orally. Inactivated vaccines must be injected.

b. Explain in some detail how typhoid fever is usually contracted? (How do most people get typhoid fever?)

Humans are the only reservoir host. They shed the bacterium in their feces, which typically contaminates water supplies. Ingestion of the contaminated water, or food washed with the contaminated water, results in disease. (fecal-oral transmission)

c. This is a live, attenuated vaccine. Explain how the pathogen is attenuated in this vaccine and why the vaccine doesn't cause typhoid fever.

An attenuated vaccine uses a "live" pathogen that is still able to replicate, but is "modified" so that it cannot cause disease. In this case, the *Salmonella typhi* bacteria used in the vaccine are mutants that do not produce a complete lipopolysaccharide (LPS). Some lipopolysaccharide is synthesized, which evokes a protective immune response.

d. This oral, attenuated typhoid vaccine stimulates both "serum antibodies" and "intestinal antibodies." What class of antibody is stimulated in the intestine? Explain your answer.

Because the vaccine is delivered through the natural route of infection, it stimulates production of IgA antibody in the intestinal mucosa.

e. People taking the Vivotif vaccine are warned against also taking sulfonamides and antibiotics. Why?

The bacteria in the vaccine are attenuated but still alive. Sulfonamides and antibiotics can inhibit their replication, which may prevent the induction of a protective immune response.

f. Why does the dead, injected, whole-cell typhoid vaccine stimulate one type of antibody, while the oral, attenuated typhoid vaccine stimulates another? (1)

Because the oral vaccine follows the natural route of infection and infects cells in the human intestine, it stimulates the production of IgA. Injection will stimulate IgG but not IgA, which is associated with mucosal tissue.

2. Another typhoid vaccine available in the U.S. is the Typhim Vi vaccine.

a. What is the antigen in this vaccine and where does it come from? (Address cell structure in your answer.)

Pieces of the polysaccharide bacterial capsule

b. What type of vaccine is this (attenuated, inactivated, or subunit)? Explain your answer.

Subunit. It contains only pieces of the bacterium, not the whole cell.