The Microbial Discovery Box

Below is an overview of the activity The Microbial Discovery Box (American Society of Microbiology, Liliana Rodriquez and Robin Patterson) to incorporate information learned from Dr. Kerkhof's presentation and subsequent discussion.

Lesson Overview

Students examine the impact of microorganisms in our daily lives.

Lesson Rationale

Microorganisms can be found everywhere. And while many students do not realize it, we could not live without microorganisms. For example, microbes in the ocean help produce the oxygen we breathe, microbes in the soil help produce the food we eat, and microbes in our digestive system help process the food that we eat and make vitamins for us. Though many students think that they are bad and cause diseases. This is any easy exercise for students to examine the impact of microorganisms in our daily life and consider their applied potential.

Key Concept

Students will examine the impact of microorganisms on our daily life and understand that microorganisms have the greatest diversity of all living organisms.

Microbial Discovery Activity

The Microbial Discovery Box

Authors

Liliana Rodriguez, MPH, RM(AAM), M(ASCP)
The University of Texas Health Science Center
School of Public Health
Houston, TX
Liliana.F.Rodriguez@uth.tmc.edu

Robin Patterson, Ed. D.
Butler County Community College
Butler, PA
robin.patterson@bc3.edu

Contributors

The concept of a microbial discovery box was first introduced in 1992 by Dr. Douglas Zook from Boston University in his book *"The Microcosmos Curriculum Guide to Exploring Microbial Space."* The idea was later expanded and presented as a hands-on activity to science teachers during the American Society for Microbiology's Microbial Discovery Institutes and Workshops that ran from 1997 through 2005. Many enthusiastic microbiologists contributed to the development of the activity as it looks today. We would like to especially thank Danny Opheim, Kenneth Anderson, T. Thomas, Stephen Wagner, and Deborah Harbour.

Intended Audience

K-4	
5-8	X
9-12	X

Activity Specifications

Classroom setting	X
Requires special equipment	
Uses hands-on manipulatives	X
Requires mathematical skills	
Can be performed individually	X
Requires group work	
Requires more than one (45 min) class period	
Appropriate for special needs student	

American Society for Microbiology
Education Department
1752 N Street, NW
Washington, DC 20036
EducationResources@asmusa.org

An Educational Program of
AMERICAN SOCIETY FOR MICROBIOLOGY

Introduction

Description

This is a relatively simple activity designed for students to examine the impact of microorganisms in our daily life.

Abstract

In this exercise, students examine the impact of microorganisms in our daily life and consider their applied potential. They can also conduct independent research and communicate their findings with the rest of the class.

Core Themes Addressed

Microorganisms and the Environment

Keywords

Microbes, Food, Vitamins, Beneficial

Learning Objectives

At completion of this activity, students will examine the impact of microorganisms on our daily life and understand that microorganisms have the greatest diversity of all living organisms. This activity is simple and easily achievable, creates enthusiasm in the classroom, and counteracts students' fear of microorganisms.

National Science Education Standards Addressed

This lesson is aligned with the following National Science Standards:

Science as Inquiry - In completion of this activity, students develop skills related to posing a scientific question. Students investigate the relationship of many daily use products to microorganisms.

Science and Technology - This activity helps students determine how microorganisms can be utilized to improve crops, deliver vaccines, and produce enzymes in large quantities.

Life Science - In completion of this activity, students will discover the behavior of microorganisms including the production of enzymes and antibiotics.

Personal and Social Perspectives - This lesson will aid students in developing understanding about natural resources (how microorganisms are beneficial to humans), and aspects of environmental.

Teacher Handout

The Microbial Discovery Box

Necessary Student Background

It will be helpful (but not required) that students have some basic knowledge about the characteristics and differences among the main groups of microorganisms, such as bacteria, viruses, fungi and algae. A good source of basic information about microorganisms is ASM's Microbe World (http://www.microbeworld.org/).

Teacher Background Information

Say the word “microbe” and immediately an image of a bad, tiny, unseen organism will come to mind for most people. The television broadcasts commercials for antibacterial soaps and sprays, the eleven o’clock news headlines the latest *E. coli* outbreak and the monthly magazines print articles on “the dirtiest places in your house (no, it's not the bathroom).” But are most microbes as bad as the press makes them out to be? Hardly. Only 1% of the bacteria that have been studied are known to cause human disease. So what about the other 99%? Would you believe that many of them are actually useful? This activity is designed to highlight the role microbes play in the production of foods, vaccine, vitamins, and many other products, as well as the important associations made by microbes that contribute to the enrichment of our lives.

Class Time

The exercise can be completed in a 50 minutes class period. Materials are distributed to students at the beginning of class. Then students "brainstorm" with their group to determine the relationship of their items to microorganisms. Some relationships will be quickly discovered during class. In all likelihood, students will need to do some research to solve the mystery.

Follow up options:

- Groups can be asked to make short presentations concerning their items during class. This can be done for a few minutes over several class meetings.
- Students may be challenged to find other items for the Microbial Discovery Box. These items can be used to stimulate discussion. When tied to bonus points, this can be quite effective

Teacher Preparation Time

Approximately one hour is initially needed for brainstorming and planning the best approach. The time to prepare a Microbial Discovery Box varies because the box can be prepared in two ways. The items can be either, collected over the summer by the teacher to have ready by the beginning of school, or the collection of items can be assigned to the students as a class project for the school year. The items collected include various things that come directly from microbes or utilize microbes (see supplementary material section).

If it's decided to involve the student's participation, they are instructed to “go on a scavenger hunt” looking for items related to microorganisms. This is not very difficult for nearly everything we can pick up, see, or find in our visible world has a direct connection to microorganisms.   For example:

- *Grocery Store Microbiology*: Students find one or two items in each aisle of a grocery store that are produced with the aid of microorganisms.
- *An Array of Antimicrobial Agents*: Students find as many examples of antimicrobial agents as possible in the grocery store, drug store, pet store, and/or beauty supply store. There are at least four different products with the Lysol brand name, each with different active ingredients! Also, the pet store is a virtual pharmacy with antibiotics galore for fish.

Materials and Equipment

There are many items appropriate for a "discovery box." These are just some examples:
- medium size plastic container or card box
- toothpaste cartons
- can of oil
- rice
- small copper tube or wire (or a penny)
- milk carton (rinsed and dried)
- empty can of green beans
- empty and clean plastic yogurt containers
- jar of vitamins
- plastic plant
- chalk
- envelope of baking yeast
- empty antibiotic prescription container
- cheese wrappers
- picture of bread (from a magazine or downloaded from the Internet)

Methods

1. Decorate the box or ask any interested students to help.
2. Place all the items in the box and locate it in a visible place in the classroom.
3. Ask a student to reach into the box to pull out a discovery box item.

4. Challenge students to determine if the item may come directly from microorganisms, utilize microorganisms, or in may be associated with microorganisms in some way. They can work independently or in groups.
5. If students are working in groups, and couldn't associate an item to microorganisms, assign research roles as necessary. For example, one person would have to contact a local university microbiology professor or industry representative who may be knowledgeable in that field, while other students visit the school library or browse the internet.
6. Ask groups of students to present their findings in front of the class during a following class period.
7. You can also tie this activity with bonus points to increase interest and participation.

There is a lot of flexibility to perform this activity. The teacher can facilitate it through active questioning (individual or groups) during class, or assign the items to individual students or groups to conduct inquiry-based investigation. Several options for modifications are listed in the Supplementary Materials section below.

Microorganisms

This exercise does not require the use of microorganisms.

Assessment

To determine if students have achieved the stated learning objectives in addition to group discussion, have students complete and hand in the student worksheet. Students can also compose an independent write up, and short quizzes can be prepared with the material provided here.

Supplementary Information

ACETONE - Prior to 1914, acetone was made by pyrolysis (dry heating) of wood. Nearly 100 tons of wood were needed to make 1 ton of acetone. When World War I erupted, the demand for acetone quickly exceeded supply. Acetone was used as a solvent for nitrocellulose in the manufacture of the explosive powder cordite. Chaim Weizmann developed a fermentation process in which *Clostridium acetobutylicum* fermented 100 tons of molasses or grain into 12 tons of acetone and 24 tons of butanol. Butanol was used in the production of artificial rubber. Manufacture of these products continued until the 1950s, when petrochemicals became cheaper to use. In 1948, Chaim Weizmann became the first president of the State of Israel.

ACIDOPHILUS MILK – is produced by inoculating skim milk with *Lactobacillus acidophilus.* This bacterium is normally part of the intestinal flora. This aids in the digestion of the milk.

AIR - About 50% of the oxygen we breathe comes from microbes in the ocean. (microalgae, autotrophic bacteria) as a result of photosynthesis by plankton

BEAN-NO - Contains alpha galactosidase isolated from the mold, *Rhizopus niger*, which breaks down the galactose polymers found in some beans. Otherwise, this polymer would be degraded in the large intestine by bacteria, producing galactose which is then degraded by intestinal microbes resulting in the production of large quantities of carbon dioxide gas, producing flatulence.

BEER - Grain, (for example barley), is allowed to germinate producing enzymes that convert polysaccharide to sugars. Hops (female flowers of *Humulus lupulus*) are added to give a bitter flavor and inhibit bacterial growth. Then sugars are converted to alcohols by *Saccharomyces cerevisiae* (top yeasts which produce more alcohol and carbon dioxide) or *S. carlsburgensis* (bottom yeasts).

BO-TOX – Botulinum A toxin is one of the most potent toxins known. This toxin is produced by *Clostridium botulinum*, and is responsible for the often fatal disease botulism. This toxin works by paralyzing muscle groups. Botox, a highly purified form of the botulinum toxin works the same way to treat individuals suffering from muscle spasms. (It is also used to paralyze muscle groups that are responsible for leading to wrinkles, thereby reducing the wrinkles.)

BREAD - The yeast *Saccharomyces cerevisiae* ferments the sugars producing alcohol, carbon dioxide (which causes the bread to rise), flavor and aromatic compounds (esters, which are alcohol and acid combinations), and changes the texture of the dough by an unknown process.

BRIE, CAMEMBERT, and other soft-ripened cheese - a fungus oxidizes the acids present in the curd thus causing the pH of the curd to rise. This provides a favorable environment for a bacterium (commensalism) which is also involved in the cheese production process. The fungus gains no advantage or disadvantage from the presence of the bacterium.

BROMELIAD - Grows at the top of trees and requires a symbiotic bacterium in order to fix nitrogen gas from the air to support growth.

BUTTER - A starter culture containing *Streptococcus lactis* (to make lactic acid) and *Leuconostoc citrovorum* is added to pasteurized sweet cream. *L. citrovorum* converts citric acid to diacetyl, giving butter its taste and aroma. This buttermilk is churned to separate the fat globule. The resulting butter is washed with salt solution and worked to distribute the water droplets uniformly.

BUTTERMILK - Formed by adding unpasteurized milk to undergo spontaneous fermentation at room temperature. Caused by the action of *Lactobacillus bulgaricus* and *Leuconostoc* in milk.

CAESAR SALAD – Historically (since the mid-1920s) the Caesar salad was a mixture of Romaine lettuce, Parmesan cheese, lemon, garlic, oil, and raw eggs. However, raw eggs are often the source of *Salmonella* infections. The USDA has now approved of a pasteurization process that destroys egg-borne *Salmonella.* The days of enjoying a true Caesar salad (or raw cookie dough) are nearly over!

CHALK - Calcium carbonate (limestone) produced from the calcareous shells of marine microorganisms (Coccolithotrophs) that have settled to the bottom of the sea.

CHEESE - Different types of bacteria and fungi are added to milk curd and are allowed to ferment and age which produces different types of cheeses with varying taste and hardness. They produce unique flavors, colors (*Penicillium roqueforti* in Roquefort cheese), and gas holes and nutty favor (*Propionibacterium shermanii*) in Swiss cheese.

CHOCOLATE - Pods containing the beans in the pulp are collected, broken open, and the seeds are removed. The beans are spread on banana leaves or put in sweat boxes where wild yeast ferment them for 2-9 days, producing various flavored compounds and acids. The beans are then dried and bagged. Cocoa is added to vanilla milk solids, and sugar to form sweet chocolate, a treat for nobility of South America.

CHOCOLATE COVERED CHERRY - amylase is put into the center of the cherries during manufacture along with doughy starch. Amylase acts to liquefy the starch by breaking it down into simple sugars which form the liquid sugary substance in the center of the cherry.

CLEANSER AND POOL FILTERS - Made of diatomaceous earth. Found in great deposits in Nevada and Arizona where inland seas with diatoms once existed. Diatoms also are used for reflective surfaces in highways, insecticides (spread on leaves where they are eaten and grind up the insect larvae's intestines), industrial filters and non-scratching cleaners.

COAL - There is increasing evidence that much of the organic material in the earth's sediments is bacterial in origin. About 90% of this material is in the form of insoluble KEROGEN, an organic precursor of petroleum. The haploid bacteriohopanetetrol (ho- pan-e-tetrol) can be isolated from kerogen. Apparently the organic matter in dead organisms is converted by bacteria into kerogen, which when treated with heat and pressure is anaerobically converted into coal and petroleum.

COCOA – derives some of its taste from the microbial fermentation that helps remove cocoa beans from the pulp covering them in the pod.

COFFEE BEANS - are grown on an evergreen shrub. The cherry contains the seeds which are enclosed in a thin skin and parchment covering, and are embedded in pulp. The pulp is removed and the coated beans are fermented to remove and wash away the pulp. The beans are then dried and roasted. Coffee was first reported in Arabia where coffee houses became so popular they were banned in 1520. In Europe coffee houses became popular and were considered houses of sedition.

CORAL - photosynthetic eucaryotic algae, *Symbiodinium microadriaticum (Gymnodidium*-like in free culture), produces carbohydrates through photosynthesis. These carbohydrates are later utilized for energy by the living coral. The carbon dioxide production allows calcium carbonate coral reefs to form.

CORTISONE CREAM - Microorganisms are used in the production of steroid hormones in a process called bioconversion; one compound is converted to another by enzymes in microbial cells. The first application of bioconversion to hormone synthesis was the use of the mold *Rhizopus nigricans* to hydroxylate progesterone. The use of this microbial step simplified the chemical synthesis of cortisone from bile acids from 37 to 11 steps. It also reduced the cost of cortisone from $200/gram to $6/gram. Subsequent improvements have further reduced the price to below $0.70/gram. Other hormones produced with the help of microorganisms include insulin, human growth hormone, and somatostatin. They are made by recombinant DNA technology using strains of *Escherichia coli.*

CRYPTOBIOTIC CRUST - symbiotic microbial communities found in dryland regions that initially form an inconspicuous grey-brown covering of the sand surface consisting of fungi, cyanobacteria and lichens, but in later stages of development the crusts form small "humps" on which mosses grow. The growth of all these pioneer organisms contributes organic matter that aids water retention and paves the way for growth of higher plants. Lichens, which consist of a fungal tissue containing either green algae or cyanobacteria as the photosynthetic partner, play a vital role in colonization of the bare sand. In this case the lichens contain cyanobacteria which fix atmospheric nitrogen (N_2) gas into amino acids and thus progressively enrich the soil with nitrogen for plant growth. The filamentous cyanobacteria also secrete a mucilaginous sheath that helps to bind sand particles together.

DAIRY EASE -This product contains lactase produced by a yeast (*Kluyveromyces lactis*) which can be added to food to degrade the lactose in dairy products. Many people, especially as they age past 45, start to become lactose- intolerant. This means that they do not produce the enzyme in the small intestine required to degrade the sugar lactose, which is typically found only in colostrums (mammalian mother's milk and other dairy products). The microbes in the large intestine degrade the undigested lactose to glucose and galactose and produce gas in the large intestine resulting in gas formation, bloating, and diarrhea.

DAVINCI'S "LAST SUPPER" - Destruction of works of art, both painting and sculpture, has usually been blamed solely on the ravages of pollution due to modern-day advances. It is now known that a microecosystem of microbes can live in stone, fed by air-borne pollutants. *Thiobacillus thioparis* uses sulfur dioxide (smog) gas and converts it to sulfuric acid which acts upon the calcium carbonate of marble to release carbon dioxide and the salt calcium sulfate (better known as plaster). The plaster is easily washed away, softening, then destroying the features of the sculpture. Fungi also can push their hyphae into the rock, splitting it making way for other bacteria and algae to penetrate and produce damaging organic acids. Frescoes, such as DaVinci's *Last Supper* are being destroyed in the same fashion. Antibiotic and chemical "therapy" has been attempted to save these valuable works of art.

DESERT VARNISH - Rugged mountain peaks and sun-baked boulders throughout the arid Southwest are often colored in beautiful shades of orange, green, yellow and gray. At first glance the colorful coatings resemble a layer of paint, but close examination reveals that this unusual phenomenon is caused by a thin layer of microscopic organisms. The organisms include colonies of bacteria called "desert varnish," and colonies of symbiotic lichens. These miniature rock dwellers have survived for countless centuries in some of the most seemingly inhospitable environments on earth and may represent some of the oldest living colonial life forms. Desert varnish is a thin coating (patina) of manganese, iron and clays on the surface of sun-baked boulders. It is formed by colonies of microscopic bacteria living on the rock surface for thousands of years. The bacteria absorb trace amounts of manganese and iron from the atmosphere and precipitate it as a black layer of manganese oxide or reddish iron oxide on the rock surfaces. This thin layer also includes cemented clay particles that help to shield the bacteria against desiccation, extreme heat and intense solar radiation. Several genera of bacteria are known to produce desert varnish, including *Metallogenium* and *Pedomicrobium*.

DYNAMITE - is made from diatomite by soaking it in the liquid explosive nitroglycerin. Diatoms are microscopic algae in the phylum Bacillarrophyte of the Protista kingdom. Yellow or brown in color, almost all diatoms are single-celled, dwelling in fresh and salt waters. Diatoms make up most of the phytoplankton of the cooler part of the ocean and are an important food source for fish and other marine animals in this region. When they die, the shells, called frustules, sink to the bottom of the body of water and fossilize. At the bottom of the sea, the frustules harden into rock called diatomite

EQUAL- Dimers of phenylalanine (made by bacteria) form the Aspartame (Nutrasweet).

FROST-FREE – "Ice-minus" bacteria are genetically engineered *Pseudomonas syringae* that lack a protein that promotes the formation of ice crystals. The idea was that if crops were sprayed with this bacterium, the bacteria that promote frost formation would be squeezed out and the crops, thereby giving some protection from frost damage. This hasn't been put into practice, however, it was a newsworthy experiment—one of the first to address the intentional release of a genetically modified organism.

GOLD - Small pieces (0.1- 0.5 mm) of gold panned in Alaska, China and South Africa apparently are *Pedomicrobium*-like bacteria that had soluble gold accumulate on or in the cell. This is called bacterioform gold and indicates a role of bacteria in gold geochemistry for half of earth's history.

GRASS, TREES, PLANTS - Mycorrhizal (endomycorrhizal) associations where fungi penetrate the plant cell forming arbuscules (fungal structures), which die and degrade, releasing nutrients to the cell. Most plants have endomycorrhizal associations.

HEPATITIS B VACCINE - Because yeasts have plasmids, they can be used for recombinant DNA applications. Insertion of the viral gene coding for a the surface antigen (HBsAg) into the plasmid of *Saccharomyces cerevisiae* results in the production of the antigen by the yeast. Purified antigen is obtained by lysing the yeast cells and separating the antigen by biochemical and biophysical techniques. Two hepatitis vaccines, Recombivax and Enginex, are currently made in this way.

HUMULIN - For years, insulin had been obtained from the pancreatic tissue of animals. This often led to allergic reactions in diabetics using the insulin. By inserting the gene for human insulin into a bacterial plasmid, the bacteria could be used as factories for the insulin, called Humulin. Since it is human insulin, allergies do not occur.

KARO SYRUP - High fructose corn syrup from glucose isomerase of bacteria and fungi produced from starch.

KEFIR - Like buttermilk, except that *Saccharomyces kefir* is added, which produces gas and alcohol, forming an effervescent drink.

KETCHUP - Contains acetic acid (vinegar) made by from ethanol by the bacterium *Acetobacter* spp.

KUMISS -Similar to Kefir but made of mare's milk in Russia near Kume River. In Colombia, kumiss is made by adding cinnamon and sugar to the sour cow milk after it has been whipped.

LACT-AID MILK (SWEET ACIDOPHILUS MILK) - contains the bacterium *Lactobacillus acidophilus* which adds the enzyme lactase to milk. Such milk may be tolerated by people who are lactose intolerant because the lactase degrades the offending lactose, which otherwise undegraded, is fermented by bacteria in the small intestine, producing gas.

LEBEN - Like yogurt, except made from goats milk in Egypt and Lebanon.

MONSODIUM GLUTAMATE - The favor enhancer, MSG, used in Asian cooking is produced by fermentation utilizing *Corynebacterium glutamicum*.

OIL GOBBLERS - About a hundred known species of bacteria and fungi are able to use oil components to sustain their growth and metabolism. In areas free of oil contaminants their proportions are about 10X lower than in areas polluted by oil.

ORCHID - orchids require a certain microorganism to thrive. This organism is a fungus that forms a symbiotic relationship (called a mycorrhiza) with the orchid roots. The fungi penetrate the root cells, and increase their absorptive abilities. Many trees, especially conifers, also have mycorrhizae, and are able to live in sandy soils where they normally would not thrive.

PARAMECIUM - hosts the green alga *Chlorella* as a symbiont. Chlorella provides organic carbon and oxygen; *Paramecium* provides protection, mobility, growth factors, and carbon dioxide.

PEANUTS, PEAS, LEGUMES – Nitrogen fixation—*Rhizobium* and *Bradyrhizobium* are associated with the clover and other legumes. *Frankia* is another group of bacteria that are associated with roots in alder bushes and other non legumes. They attach to the root hairs and infect them forming infection threads, these infected cells and bacteria convert into bacteroids, resulting in enlarged root hairs that fix nitrogen gas.

PENNY - Copper is mined with the use of bacteria that convert the ferrous ion to the ferric ion. The ferric ion reacts chemically with copper ore to produce soluble copper sulfate. *Thiobacillus ferroxidans* then converts the ferrous form back to the ferric form of iron (Fe^{+2} to Fe^{+3}).

PICKLES, OLIVES - *Leuconostoc* spp and *Lactobacillus* spp produce lactic acid from plant sugars which must be squeezed out of the plant cells before bacteria can utilize them. Salt is added to give favor, inhibit bacterial growth, and bring out the favor from plant juices. Esters and diacetyl are also formed and give flavor and aroma. In pickles 20% salt is used and only *Lactobacillus plantorium* can grow.

PINE, OAK TREES - *Mycorrhizae* associations. The growth of plants is aided in an enormous manner by the symbiotic interaction with fungi associated with their roots. Ectomycorrhzae (a fungal mantle formed over the roots-no vesicles or arbuscules formed) are used for the growth of commercial pine trees.

PLASTIC - *Alcaligenes eutrophus* is a bacterium that, under the right conditions, makes plastic.

POI - A natural fermentation of the stems of steamed, ground taro roots for a period of 1-6 days. There is a succession of bacteria which is finally dominated by yeasts and *Geotrichum candidum*. The final product contains a variety of acids, ethanol, and carbon dioxide.

RAIN-FRESH SMELL – Ever noticed that distinct smell in the air after a rainstorm? That pleasant rain-smell is actually due to a filamentous bacterium known as *Actinomycetes*. *Actinomycetes* grows in soil when conditions are damp and warm. When the soil dries out, the bacteria produce spores in the soil. The wetness and force of rainfall kick these tiny spores up into the air where the moisture after a rain acts as an aerosol (just like an aerosol air freshener). The moist air easily carries the spores to us so we breathe them in. These spores have a distinctive, earthy smell we often associate with rainfall.

RICE - Requires blue green algae *Anabaena azollae* which grows on *Azolla* (a little floating plant which grows in rice fields) and fixes nitrogen which is required for the repeated growth of rice in fields without fertilizer

SAKE - Produced by the treatment of rice with *Aspergillus oryzae* which produces a starch degrading enzyme that releases sugars from starch. Then yeast including *Saccharomyces sake* is added to produce rice wine that contains alcohol at 14-24%.

SALAD DRESSING - Is made with vinegar, which is acetic acid produced by the fermentation of ethanol (alcohol) by a bacterium, *Acetobacter* spp.

SAUERKRAUT, KIMCHI - Natural fermentation of cabbage by salt tolerant, lactic acid bacteria *Lactobacillus* and *Leuconostoc*. Cabbage is salted with sodium chloride and crushed (to remove oxygen) in a saline solution. The natural lactic acid bacteria produce lactic acid which prevents other bacteria from growing.

SILAGE - Non degradable plant material produced in a silo (a giant fermenter). Plant matter is collected by the farmer, cut into small pieces and tightly packed in a silo (to make it anaerobic). There it ferments due to natural *Lactobacilli*, producing lactic acid and heat (other bacteria produce acetic acid), which prevents the growth of other microorganisms and the destruction of the stored plant materials. Silage is not green but contains almost ail of the nutrients of fresh material and animals love its flavor.

SOFT SCRUB - Calcium carbonate from carbonate shells of microbes.

SOY SAUCE - A complex three step fermentation of soy beans, wheat, and bran by bacteria and fungi. The critical fermentation is with *Aspergillus oryzae* which produces a black compound which is secreted into the sauce.

SPIRULINA, CHLORELLA - Cyanobacteria or green algae produce vitamins and proteins, and are thought to be a health food.

SWEETENER - Sucrose is the common form of sweetener that we isolate from sugar beets and cane sugar. Invertase (Sucrase) is a yeast derived enzyme that converts sucrose to glucose and fructose. However, we can also take starch (isolated from a variety of plant products) and convert it to glucose with amylase. The glucose is then converted to fructose, which also tastes very sweet but has one half the calories of sucrose. The enzyme that accomplishes that conversion is glucose isomerase and is isolated from a bacterium. The product is called corn syrup or fructose sweetener.

SWEAT SOCKS—NOT SWEET SOCKS – Smelly feet have been a socially awkward part of our society for a long time. Sweaty feet are more likely to be to have an offensive odor, but it isn't the sweat, which is mostly salt and water, that causes the distinctive smell. Rather it is the bacteria that live on the feet that lead to the smell. The bacteria enjoy the dark, damp environment of a sweaty sock, and have a feast, growing and metabolizing the sweat. When you remove the sock, the bacterial byproducts are responsible for the odor.

SWIMMING POOL FILTERS - Silicates mined from an ancient sea bed in the southwest US where the bodies of silicates (diatoms) are deposited a thousand feet thick. The ancient lake was Manly Lake, south of Death Valley.

TEMPEH - An Indonesian food prepared from soybeans by inoculating soaked, dried beans with *Rhizopus* spores. After fermenting for 20 hours, it is salted and cut for eating.

TIDE or CHEER - Protease from *Bacillus subtilis* or *B. licheniformis* and sometimes amylase to remove cold-soluble laundry starch.

TOFU - A fermented product of soybeans in which the beans are soaked, ground, curdled with calcium or magnesium salts, and fermented with *Mucor* species for one month.

TYPHUS—OR IS IT? During the time of the Nazi occupation of Poland, two physicians, Lazowski and Matulewicz, took advantage of a bacterium called Proteus 0X19 to fool the Nazi's into thinking their little town in Poland was experiencing an epidemic of the highly contagious disease, typhus. Many diseases are diagnosed by looking for an antibody response to the organism in question. Oddly enough antibodies directed against the typhus agent will react against the *Proteus* organism. Likewise, antibodies directed against Proteus produce anti-typhus antibodies. These doctors injected the townspeople with Proteus and submitted the blood for testing. The Nazis were so fearful of the infection that they stayed out of the town.

VINEGAR - A product made by dripping ethanol over wood chips covered with *Acetobacter* which get their energy by converting ethanol to acetic acid (vinegar).

VITAMINS - Nutritional products of yeast cells and bacterial fermentations made into tablets and powders, which are products for human and animal consumption. Bacteria in the intestine provide us with half of the Vitamin K that we need.

WINES - Sugars in red and white grape juices are fermented by *Saccharomyces cerevisiae* to produce alcohol for red and white wines.

YEAST PACKET - Contains dried *Saccharomyces cerevisiae* which is stable to drying due to high content of trehalose. Can be used as a source of vitamins, for making bread (carbon dioxide formation that causes bread to rise), or alcoholic fermentation.

YOGURT - Milk fermentation at a high temperature with *Streptococcus thermophilus,* and *Lactobacillus bulgaricus*. Lactic acid which lowers the pH, prevents other microbes from growing.

POSSIBLE MODIFICATIONS

There are many variations to use the Microbial Discovery Box in the classroom. Here are just a few examples:

- Introduce the items during lecture time to stimulate interest and questions. They easily can be used, singly or grouped, to introduce concepts or stimulate discussion.

- Provide a box in the classroom or lab and ask students to bring in items. Ask the student to give an oral report about the item's relationship to microbes and give points before they can add it to the box.

- Ask groups of students to “go on a scavenger hunt” looking for items that have a particular microbe or microbial group in common (for example: *Saccharomyces*, *Bacillus*, diatoms).

- Ask students to prepare a menu for a restaurant in which all foods are produced or prepared with the assistance of microbes. Cheese, yogurt, beer, and wine come to mind easily… encourage them to think about industrial applications, such as enzyme production, and their menus will expand beyond the obvious.

- Prepare some mini-boxes with 4-5 items each, distribute to students, and ask them to determine which one of the items is not like the others. **See Appendix 1.**

- Combine the Microbial Discovery Box activities with reading assignments using the Intimate Strangers Book. **See Appendix 2.**

Student Handout

The Microbial Discovery Box

Introduction

Despite the fact that we can't see them without the aid of special magnification, microorganisms dominate our planet. Almost all processes necessary to sustain life on earth are at least partially dependent on microbes. They have the greatest diversity of all living organisms, and we now look at them as a wealth of untapped resources. In this exercise you will begin to discover the role that this "unseen" world plays around us.

Necessary Student Background

Review the Meet the Microbes section in MicrobeWorld at http://www.microbeworld.org/ to learn the main characteristics of the different kinds of microorganisms.

Vocabulary

Include a list of new terms the student will encounter and their definitions.

Materials Checklist

Microbial Discovery Box located in your classroom.

Procedure

1. Each group of students will be assigned 4 items from the Microbial Discovery Box by the teacher. These items may come directly from microorganisms (or microbes), utilize microorganisms, or in some be associated with microorganisms.

2. Brainstorm with your group to determine what your items have to do with microbes.

3. Record your thoughts below.

4. Your teacher will instruct you on the follow up options if the answers are not found during class.

Item	**How is this item related to microbes?**

Appendix 1

The Microbial Discovery Box

MicroDiscovery Box #1

Contents:
License plate, dynamite (picture or cartoon), cleanser, swimming pool (picture or cartoon), and cortisone cream.

Answer: All but the cortisone cream have DIATOMS in common.

Diatoms are microscopic algae in the phylum Bacillarrophyte of the Protista kingdom. Yellow or brown in color, almost all diatoms are single-celled, dwelling in fresh and salt waters. Diatoms make up most of the phytoplankton of the cooler part of the ocean and are an important food source for fish and other marine animals in this region. It is not unusual for one gallon of seawater to contain 1-2 million diatoms. Diatoms have hard "shells" made from silica that they extract from the water. When they die, the shells, called frustules, sink to the bottom of the body of water and fossilize. At the bottom of the sea, the frustules harden into rock called diatomite. One of the most famous and accessible diatomites is the Monterey Formation along the coast of central and southern California. Diatomite is also called "diatomaceous earth". It is white or cream-colored, chemically inert and has a rough texture. It is suitable as a filtering agent (swimming pool filtration), an abrasive polish (cleanser, toothpaste), an insulator for temperature and sound (such as firebrick), an ingredient in pharmaceutical preparations, and an additive to paint to increase the night visibility (signs and license plates). Dynamite is made from diatomite by soaking it in the liquid explosive nitroglycerin.

Cortisone cream does not belong with this group but microorganisms are used in the production of steroid hormones. This process is called bioconversion, a reaction in which one compound is produced by another by enzymes in microbial cells. The first application of bioconversion to hormone synthesis was the use of the mold *Rhizopus nigricans* to hydroxylate progesterone. The use of this microbial step simplified the chemical synthesis of cortisone from bile acids from 37 to 11 steps. It also reduced the cost of cortisone from $200/gram to $6/gram (~2003). Subsequent improvements have further reduced the price to below $0.70/gram. Other hormones produced with the help of microorganisms include insulin, human growth hormone, and somatostatin. They are made by recombinant DNA technology using strains of *Escherichia coli.*

MicroDiscovery Box #2

Contents:
Beer bottle, slice of bread, Hepatitis b vaccine (picture), vitamins bottle, penny.

Answer: All but the penny has *Saccharomyces* yeast in common.

Yeasts are unicellular fungi. These organisms often utilize alcoholic fermentation in metabolism, producing carbon dioxide and ethanol. In bread baking, *Saccharomyces cerevisiae*, is added to moistened flour which contains amylases that release maltose and sucrose from starch. The yeast produce maltase, invertase and zymase enzymes with degrade the sugars, releasing carbon dioxide, causing the dough to raise. By using more complex assemblages of microorganisms, bakers can produce special breads, such as sourdough. The yeast usually employed in beer fermentation is one of two species of *Saccharomyces*. *S. cerevisiae* gives a dark cloudiness to the beer and is carried to the top of the fermentation vessel by the foaming of the carbon dioxide. It is therefore called a "top yeast" and produces ales, porters, and stouts. *S. carlsbergensis* ferments more slowly and produces a clearer, lighter beer having less alcohol. The yeast settles out and is termed a "bottom yeast". Its product is pilsner or lager beer. Almost 75% of the beer produced in the world and most American beers are lager beers. Fermentation wastes from breweries contain significant quantities of vitamins, proteins and carbohydrates. Some breweries dispose of these wastes by drying them and selling them as supplements to animal feed. Yeasts can also be grown specifically for the vitamins they produce. S. *uvarum* produces ergosterol, a sterol that can be converted to vitamin D by ultraviolet radiation. Because yeasts have plasmids, they can be used for recombinant DNA applications. Insertion of the viral gene coding for the surface antigen (HBsAg) into the plasmid of S. *cerevisiae* results in the production of the antigen by the yeast. Purified antigen is obtained by lysing the yeast cells and separating the antigen by biochemical and biophysical techniques. Two hepatitis vaccines, Recombivax and Enginex, are currently made in this way.

The penny does not belong with the group but it is made of copper which can be mined with the help of the chemolithotroph *Thiobacillus ferrooxidans.* The bacterium obtains energy as it uses atmospheric oxygen to oxidize the sulfur attached to copper sulfate in the soil. The sulfur is oxidized to sulfate but the copper is unused and can be washed out with water.

MicroDiscovery Box #3

Contents:
Laundry detergent, drain cleaner, Lact-Aid milk, Beano, orchid (picture or plastic).

Answer: All but the orchid have enzymes produced by bacteria.

With a few exceptions, such as the extraction of the meat tenderizer papain from papaya fruit, industrial enzymes are made by microorganisms. Of the commercially-available enzymes, proteases and amylases predominate. Some laundry detergents contain proteolytic enzymes produced by the bacterium, *Bacillus.* Proteolytic enzymes have also been added to drain cleaners, where they are especially useful in degrading hair. Lipase enzymes are added to kitchen drain cleaners to degrade fats from food and cooking. Lact-Aid milk contains the bacterium *Lactobacillus acidophilus* which adds the enzyme lactase to milk. Such milk may be tolerated by people who are lactose intolerant because the lactase degrades the offending lactose, which otherwise undegraded, is fermented by bacteria in the small intestine, producing gas.

The commercial product Beano, produced by and collected from the fungus *Aspergillus niger,* contains an alpha-galactosidase, a sugar-digesting enzyme that people lack. This enzyme breaks the alpha linkages in complex sugars, such as those found in beans, before they reach the colon. Normally these sugars would be degraded by bacteria in the colon, releasing gas.

The orchid does not belong with this group, but is does require a certain microorganism to thrive. This organism is a fungus that forms a symbiotic relationship, called a mycorrhiza, with the orchid roots. The fungi penetrate the root cells, and increase their absorptive abilities. Many trees, especially conifers, also have mycorrhizae, and are able to live in sandy soils where they normally would not thrive.

MicroDiscovery Box #4

<u>Contents</u>:
Coffee can (or coffee bean), soy sauce envelope, nail polish remover (empty container), empty yogurt container, chalk.

<u>Answer</u>: All products but the chalk are produced by microbial fermentation.

Microbial fermentations are responsible for the flavor and texture of a number of foods. Fermented milk products include cheese, buttermilk, sour cream, kefir, koumiss, cottage cheese and yogurt. Yogurt is made by adding *Streptococcus thermophilus* and *Lactobacillus bulgaricus* to milk. The unique flavor is due to lactic acid and acetaldehyde made by these bacteria. The lactic acid lowers the pH of the milk, coagulating and thickening the product. The fruit of the coffee plant contains the seed or bean. The pectin is removed from the fruit through fermentation by *Erwiniadissolvens* and the bean is then roasted. A similar process is used for the cacao bean in making chocolate.

Soy sauce is made by treating a salted mixture of crushed soy beans and wheat with the mold *Aspergillus oryzae,* which degrades the starch to fermentable glucose. The product is mixed with and equal amount of salt and fermented for 8 to 12 months. The fermenting organisms are mainly the bacterium *Pediococcus soyae* and the yeasts *Saccharomyces rouxii* and *Torulopsis* species. After fermentation, the liquid part is removed and is bottled as soy sauce. The solid remains may be used as animal feed.

Prior to 1914, acetone was made by pyrolysis (dry heating) of wood. Nearly 100 tons of wood were needed to make 1 ton of acetone. When World War I erupted, the demand for acetone quickly exceeded supply. Acetone was used as a solvent for nitrocellulose in the manufacture of the explosive powder cordite. Chaim Weizmann developed a fermentation process in which *Clostridium acetobutylicum* fermented 100 tons of molasses or grain into 12 tons of acetone and 24 tons of butanol. Butanol was used in the production of artificial rubber. Manufacture of these products continued until the 1950s, when petrochemicals became cheaper to use. In 1948, Chaim Weizmann became the first president of the State of Israel.

Chalk, although not produced by fermentation, is microbial in origin. It is made of calcium carbonate (limestone) produced from the shells of dead, sedimented marine organisms called coccolithotrophs.

MicroDiscovery Box #6

<u>Contents</u>:
Moldy bread (in plastic bag or a picture), chicken (picture), bone (picture), leather belt, cantaloupe (picture), a small bag of rice.

<u>Answer</u>: All but the rice are sources of antibiotics.

Moldy bread was used to treat illness in ancient Egypt. The mold, *Penicillium*, produces the antibiotic penicillin, discovered by Alexander Fleming. In the early 1940s Howard Florey and Ernst Chain left Britain, who was heavily invested in World War II at the time, bringing a penicillin-producing culture of *P. notatum* to the USA. Robert Coghill, of the USDA, offered his assistance in devising methods to increase the yield of the antibiotic from the mold. His search led to the discovery another species, *P. chrysogenum*, isolated from a rotten cantaloupe in a supermarket in Peoria, III. Treatment with UV light mutated this organism to produce yet higher yields. By 1943, the U.S. was producing enough penicillin for the allied forces and by 1945, enough was available for the civilian population.

The source of the actinomycete which produces the antibiotic streptomycin was the throat of a chicken. It was discovered by Selman Waksman and Albert Schatz. A broken bone led to the discovery of the antibiotic bacitracin, one of the antimicrobials found in Neosporin. A girl named Tracy sustained a compound fracture. A diagnostic culture of the wound yielded the antibiotic-producing *Bacillus* bacteria.

Leather can be dyed with prontosil. Gerhardt Domagk discovered that this dye, which has no antimicrobial properties *in vitro*, does indeed have such properties *in vivo*. When introduced into an animal or human, prontosil is degraded into sulfanilimide, a sulfa drug. Rice plants thrive without the addition of fertilizers with assistance from the cyanobacterium *Anabaena,* which grows on the aquatic fern Azolla. Before planting rice, the farmer allows the flooded field to overgrow with the fern. After planting, the rice overtakes the fern, which dies and releases the nitrogen for use by the rice.

MicroDiscovery Box #6

Contents:
Cow (picture or cartoon), pine branch, termite (picture), lichen, beans, humulin (picture).

Answer: All but Humulin benefit from a symbiotic microbial relationship.

The protozoan-termite relationship is an example of mutualism, a type of symbiosis in which both organisms benefit and are metabolically dependent upon each other. The flagellated protozoa that inhabit the gut of the termite metabolize the cellulose from the wood. Termites lack the enzymes to do this and would not survive without the protozoa. Another example of mutualism is provided by lichens. These are associations between ascomycete fungi and certain genera of cyanobacteria or algae. Lichens can be used as a bioindicator of air quality.

Members of the genus *Rhizobium* are the most important symbiotic nitrogen fixing bacteria. These bacteria associate with legumes (peas, beans, peanuts, clover, alfalfa) and form nodules on the roots. Nitrogen fixation takes place in the anaerobic environment of the nodule. The nitrogen diffuses out into the root environment where it is used by the plant for protein synthesis. Many trees, especially conifers are able to live in sandy soils where they normally would not thrive due to a relationship with fungi which penetrate the roots, increasing their absorptive properties. This symbiotic relationship is called a mycorrhiza. Cows are ruminants, a group of herbivorous animals that have a stomach divided into four compartments and chew a cud of regurgitated, partially digested food. This method of feeding has evolved in animals (deer, sheep, giraffes, camels) that need to eat large amounts of food quickly with chewing being done at a more comfortable or safer location. Ruminants lack the ability to produce the enzyme, cellulase, yet eat a diet primarily of cellulose. The rumen, one of the compartments of the stomach, contains a large microbial population consisting of anaerobic bacteria, fungi, and protozoa which partially digest and ferment the food.

For years, insulin had been obtained from the pancreatic tissue of animals. This often led to allergic reactions in diabetics using the insulin. By inserting the gene for human insulin into a bacterial plasmid, the bacteria could be used as factories for the insulin, called Humulin. Because it is human insulin, allergies do not occur.

Additional Reading and Resources

An excellent resource for discovering more is Power Unseen : How Microbes Rule the World by Bernard Dixon (1994) (ISBN 0-7167-4504-6).

Fundamentals of Microbiology, I. E. Alcamo. Benjamin-Cummings. NY. 1994

Microbiology, Prescott, Harley & Klein. Wm. C. Brown: Dubuque, IA. 1990.

Microbiology - A Human Perspective, Nester, Roberts & Nester. Wm. C. Brown: Dubuque, IA. 1995

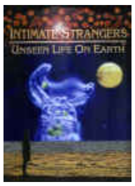
Microbiology - Principles and Application, J. G. Black. Prentice-Hall: Englewood Cliffs, NJ. 1993

The Handy Science Answer Book, The Carnegie Library of Pittsburgh. Visible Ink Press: Detroit, MI. 1994

Appendix 2

The Microbial Discovery Box

The Microbial Discovery Box activities can be combined with reading assignments from the Intimate Strangers Unseen Life on Earth Book This richly illustrated book, accompanies the PBS science documentary Intimate Strangers: Unseen Life on Earth



(http://www.pbs.org/opb/intimatestrangers/) and combines vivid, descriptive images from the series with the compelling story of microbes and their role in the Earth's ecosystem. The book offers a more comprehensive view of our relationship with the planet's tiniest inhabitants. Targeted to a general audience, the book's lively style will engage parents and their children and teachers and their students, putting the vitally important role of the microbial world into stories and terms familiar to the reader. Intimate Strangers: Unseen Life on Earth is available for $39.95. To order, call 1-800-546-2416 (U.S. and Canada) or 1-703-661-1593 (all other countries).

Readings can be organized to follow the main core concepts covered in the video series; Microorganisms as Keepers of the Biosphere (1), The Tree of Life (2), Dangerous Friends and Friendly Enemies (3), and Creators of the Future (4).

Readings	Page	Core Theme	Microbial Discovery Box Items
Cycling through the biosphere	5	1	Brie, camembert cheese, bromeliad, coral, cryptobiotic crust, desert vanish, grass, trees, plants, orchid, *Paramecium spp.*, peanuts, peas, legumes, pine or oak trees, rice, air, chalk, cleanser and pool filters, coal, Davinci's last supper, dairy ease, dynamite, gold, penny, silage, soft scrub, vitamins.
Into the Rainforest	18		
Oceans of Microbes	26		
Life in the Deep Sea	36		
The Sum of All Parts	42		
Shaking the Tree	46	2	DNA key chain, DNA structure drawing
Placing the Microbial Branches on the Tree	56		
Using DNA to build the Tree	72		
Evolution on Fast Forward	82		
Forward to the Past	86		
Living in a Microbial World	90	3	Moldy bread, cantaloupe, chicken, broken bone, leather, Lincoln, sewage, tempeh, Tide detergent, tofu, vinegar, vitamins, wines, yeast packet, yougurt.
When Friends Turn Against Us	93		
Familiar Enemies	100		
The Most Lethal of Strangers	108		
Protecting Our Territory	118		
Our battle to Control Infectious Diseases	128		
Our Evolutionary dance with the Microbes	134	4	Acetone, beer, Beano, bread, butter, buttermilk, cortisone cream, cheese, chocolate, chocolate-covered cherry, coffee beans, Equal, Hepatitis B vaccine, Humulin, Karo syrup, Kefir, Ketchup, Kumiss, Lact-aid milk, leben, monosodium glutamate, pickles, olives, poi, sake, salad dressing, sauerkraut, soy sauce, spirulina, strawberry jelly, sweetener.
Combating Antimicrobial Resistance	138		
Cleaning House	151		
Feeding the World	163		
Walk on the Dark Side	174		
Of Microbes and Humans	176		