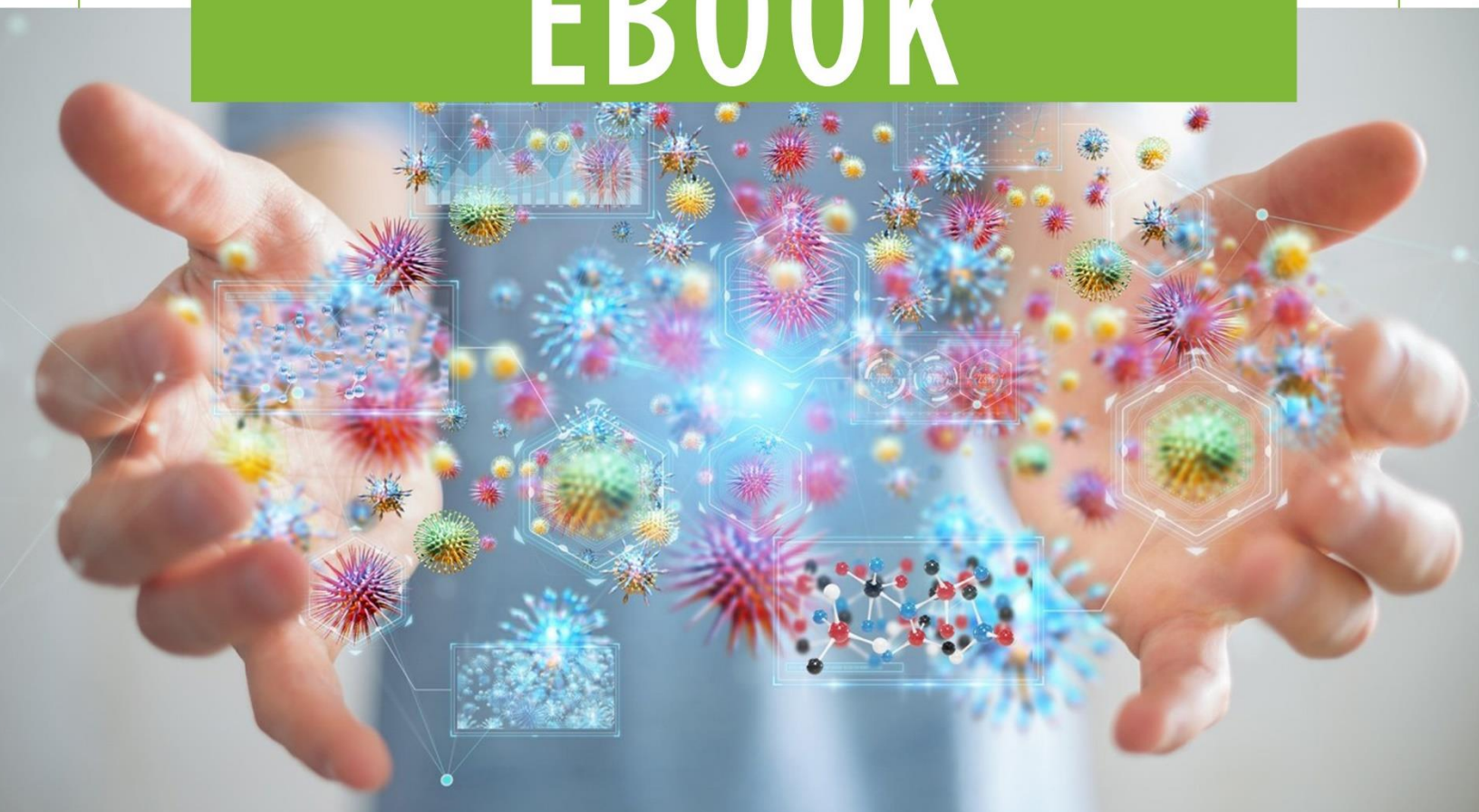
EVVIVA SCIENCES

THE AMAZING AGAR EBOOK



Thank You for Your Support!

Thank you so much for trying out one of our Agar Plate Kits. We made this E-Book to complement your experience and give you more information about microbiology. This E-Book includes exciting information about microorganisms including fun facts about bacteria, yeast and other fungi. In addition, it has more details about experiments you can perform using your kit. Lastly, it has interesting information about the life cycle of one of the most interesting and delicious types of fungus around: mushrooms. Thanks again, and contact us anytime at support@evvivasciences.com if you have any questions whatsoever!

Contents

Introduction: Microorganisms 101

The world is filled with microscopic organisms (microorganisms or microbes) including bacteria, fungus, and other species. It is said that a single teaspoon of soil contains 1 billion bacteria, and there are more microorganisms on a person's hand than there are people on the planet! Many of these life forms have a bad reputation because some can cause diseases such as pneumonia, tuberculosis, athlete's foot, and strep throat. What many people do not realize is that most microbes do not cause disease (less than 5% do), and many of these organisms play important roles in our daily lives! Here are some interesting facts about microorganisms:

- At least half of the oxygen we breathe is produced by microorganisms!
- Many medications come from bacteria and fungi. Here are just a few examples:
  - Botox, which is used to treat wrinkles, comes from the bacterium Clostridium botulism.
  - Genetically engineered bacteria can be made to produce medications such as insulin and erythropoietin (EPO).
  - Many antibiotics, which are medicines used to treat bacterial infections, are actually made by other bacteria and fungi. For example, Penicillin comes from the Penicillium fungus.

- In the average household, it is said that the bathroom is cleaner than the kitchen. Test this for yourself using your agar plate kit!
- It is said that soap and water are more effective at killing germs than hand sanitizer. You can also use your kit to test this for yourself!
- It was long thought that stomach ulcers were caused by stress and spicy foods, but we now know that they are actually caused by a bacterium called Helicobacter pylori.
    - Dr. Barry Marshall actually ate the contents of a petri dish containing Helicobacter pylori to prove that this bacterium causes ulcers. He indeed gave himself ulcers but he also won the Nobel Prize in Medicine for this discovery in 2005.
- It is said that drying your hands with a paper towel after washing will reduce bacterial counts by 45 - 60%. However, it is also said that using a hand dryer may sometimes increase the bacteria on your hands by up to 255% because it blows out bacteria already living in the conveniently warm and moist environment.
- Honey is a natural reservoir for the Botulism bacteria (the same bacteria that makes Botox). Adults can normally process it but infants often cannot, and this is why honey can be toxic to babies.
- Treating patients with antibiotics may kill "good bacteria" in the intestines, leaving room for more dangerous bacteria to start growing. This can lead to a massive infection and inflammation of the intestines known as Clostridium difficile colitis.
- Not all fungi are small. The largest living organism ever discovered is a honey mushroom in eastern Oregon, which spreads through the roots of trees and covers 2,200 acres (3.5 miles)! It is estimated to be over 2000 years old!
- It is said that chocolate has an antibacterial effect in the mouth and protects against tooth decay. I'm not sure the dentist would agree with this, but you can test the effects of chocolate on bacteria using your kit.
- When two people kiss, it is said that they exchange between 10 million and 1 billion bacteria. Bacteria can be genetically engineered to express other animal's genes. For example, fluorescent bacteria can be made from jellyfish genes. See the figure below!

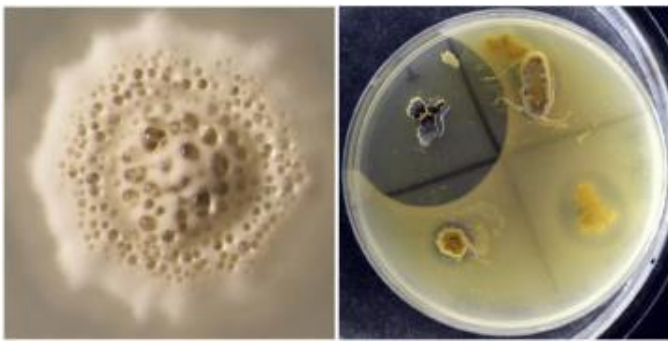


Figure: The picture on the left shows a colony of actinomycetes bacteria, which produce antibiotics used to treat patients with infections. The picture on the right shows a petri dish with bacteria growth all over except in the upper left hand corner because of an antibiotic producing microorganism.

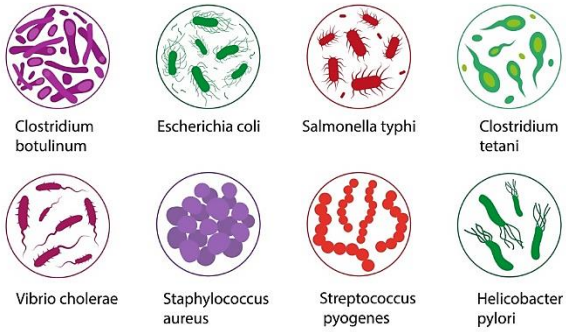


Figure: The image above shows some of the types of bacteria that cause infections and disease in humans.

Figure: The picture on the right is an x-ray of a patient with tuberculosis, an aggressive bacterial infection of the lungs. The red regions show the location of the tuberculosis bacteria in this patient's lungs.

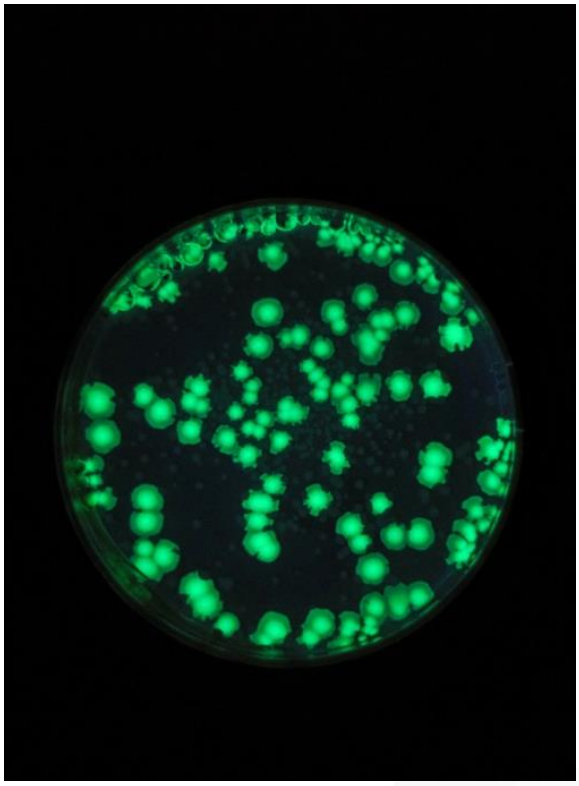


Figure: Escherichia coli or E. coli, a type of bacteria in our intestines, can be genetically engineered to express jellyfish genes. The left above shows E. coli engineered to express GFP (green fluorescent protein), a fluorescent protein produced by the jellyfish, Aequorea victoria.

Yeast!

As mentioned, not all microbes are bad! In fact, people have been using many microorganisms for the benefit of society for years. One of these microbes, which you are certainly familiar with, is yeast.

Yeasts are single-celled microorganisms that are a type of fungus. It is thought that the first yeasts originated hundreds of millions of years ago. Amazingly, there are many different types of yeast. In fact, it is estimated that there are more than 1500 species!

As mentioned, yeast are single celled organisms, which means that each yeast is just made up of one cell. In contrast, the average adult human is multicellular and has over 35 trillion cells. Interestingly, individual yeast cells can stick together and can sometimes act like a multicellular organism. For example, yeast can form strings of connected budding cells, which are called pseudohyphae. Another really interesting fact is that yeasts can have a wide range of different sizes. Some of them are only 3 µm (micrometers) in diameter. One micrometer is the length you would get if you took one meter and divided it by 100,000. However, other yeasts can grow up to 40 µm in size.

Baker's yeast, which are included in some of our kits, are a species of yeast called Saccharomyces cerevisiae. When baker's yeast feed on carbohydrates, they produce carbon dioxide and alcohol. This process is called fermentation, and people use this fermentation to produce alcoholic beverages. The carbon dioxide produced by baker's yeast is what makes bread rise!

Yeast is commonly used by laboratory researchers to understand the biology and genetics of other organisms including humans. Yeasts are small and easy to grow in a laboratory, which makes them ideal for science experiments and research. In addition, unlike bacteria, yeast cells have multiple DNA chromosomes similar to human cells, which makes them an excellent model to study. Yeasts can be genetically modified to produce chemicals and proteins that can be used to treat human illnesses. For example, yeast can be engineered to produce insulin for diabetes and can be engineered to help make vaccines for hepatitis. Amazingly, yeasts have recently been used to generate electricity in microbial fuel cells and to produce ethanol for the biofuel industry. What an amazing microbe!

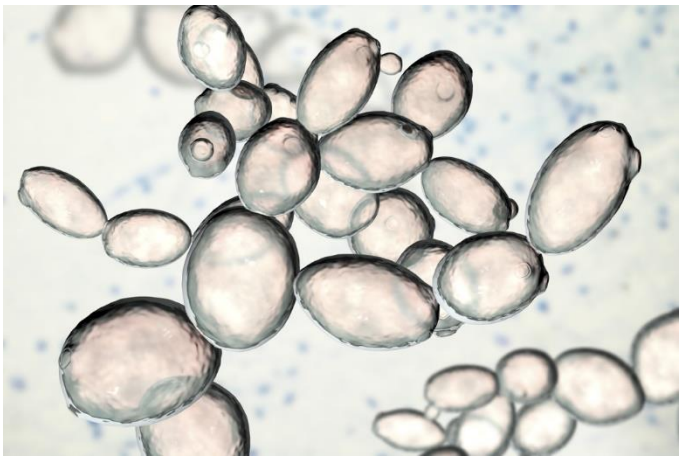


Figure: The image above is a 3D illustration of Baker's Yeast, which are also known as Saccharomyces cerevisiae. Even though yeast are single celled organisms, they can stick together in strings of connected budding cells called pseudohyphae.

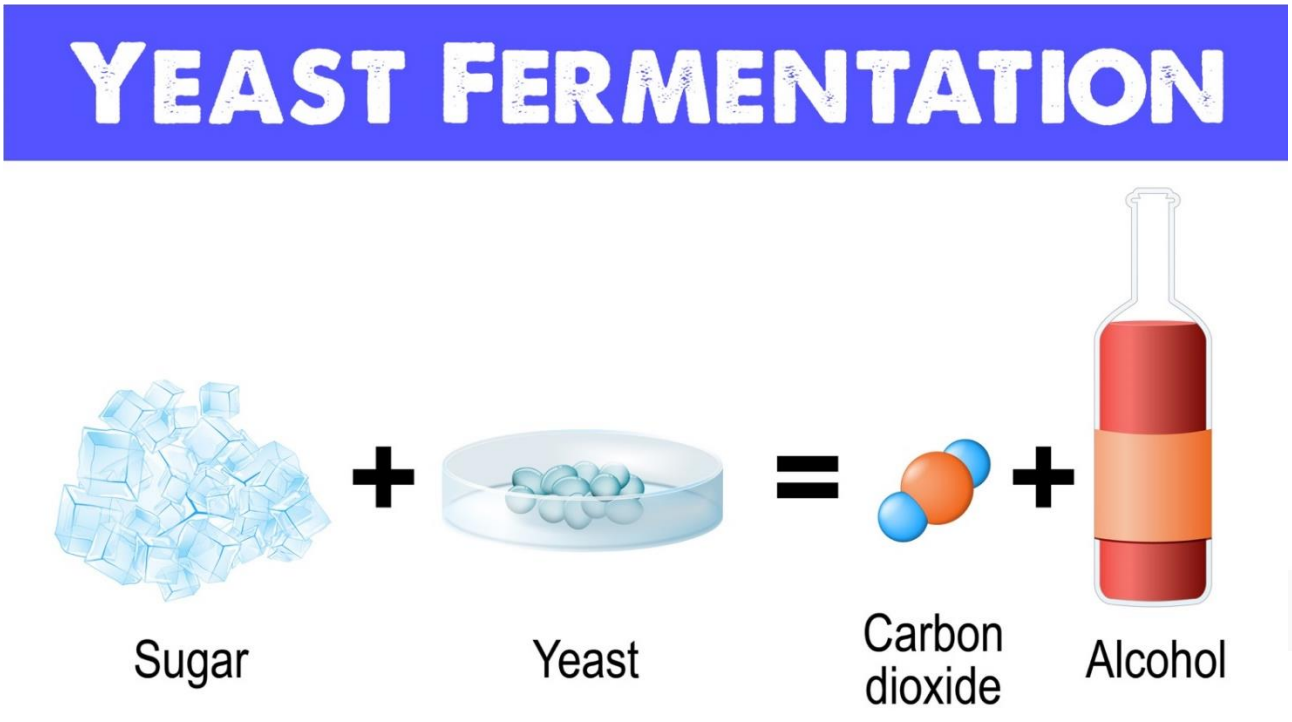


Figure: The image above is a cartoon representation of yeast fermentation. When yeast feed on sugars, they produce carbon dioxide and alcohol. The alcohol can be used to make alcoholic beverages (Brewer's Yeast). The carbon dioxide can be used to make bread rise (Baker's Yeast).

Mushrooms!

Like yeast, mushrooms are also a type of fungus, but their growth and life cycle is more complicated (see figure below). Have you ever noticed that some types of mushrooms sometimes just pop up overnight outside in the soil? If you think about it, this is incredibly fast growth, and it is where the expression "to mushroom" or "mushrooming" (which means to grow or expand quickly) comes from. What actually happens is that mushrooms may take several days to form small “fruit bodies” but once this happens, they can grow very rapidly as they absorb moisture.

Figure: A mushroom fruitbody sprouting overnight, which is where the expression “mushrooming” (which means to grow or expand quickly) comes from.

The fruitbody of a mushroom is the familiar stalk and cap of a fully grown mushroom. However, first the mushroom forms a smaller fruiting body, which is known as a mushroom pin because of their small size. As the pin gets slightly bigger, it is then called a button. Once it reaches the button stage, now the mushroom can grow really fast into a mature fruitbody, simply by absorbing water rapidly from its surroundings. Basically, the water quickly inflates the mushroom cap, which is how mushrooms quickly sprout overnight at times. Amazingly, there are some mushrooms such as Parasola plicatilis, that grow quickly overnight and then sometimes disappear by late afternoon on a hot day after rainfall. The warm weather basically causes the mushroom cap to lose water, and the mushroom shrinks away in the heat.

Once the fruiting body of a mushroom is formed, it often releases mushroom spores into the environment. Spores can stay dormant for quite some time. However, under the right conditions, which usually involves exposure to water or moisture, the spores will germinate. As they grow, they will eventually form a web of fibers (usually white in color) called mycelium. In fact, some of our kits include oyster mushroom mycelium.

Though mushroom fruiting bodies are short-lived, the underlying mycelium can itself be long-lived and massive. A colony of Armillaria solidipes (formerly known as Armillaria ostoyae) in Malheur National Forest in the United States is estimated to be 2,400 years old, possibly older, and spans an estimated 2,200 acres (8.9 km2).[15] Most of the fungus is underground and in decaying wood or dying tree roots in the form of white mycelia combined with black shoelace-like rhizomorphs that bridge colonized separated woody substrates

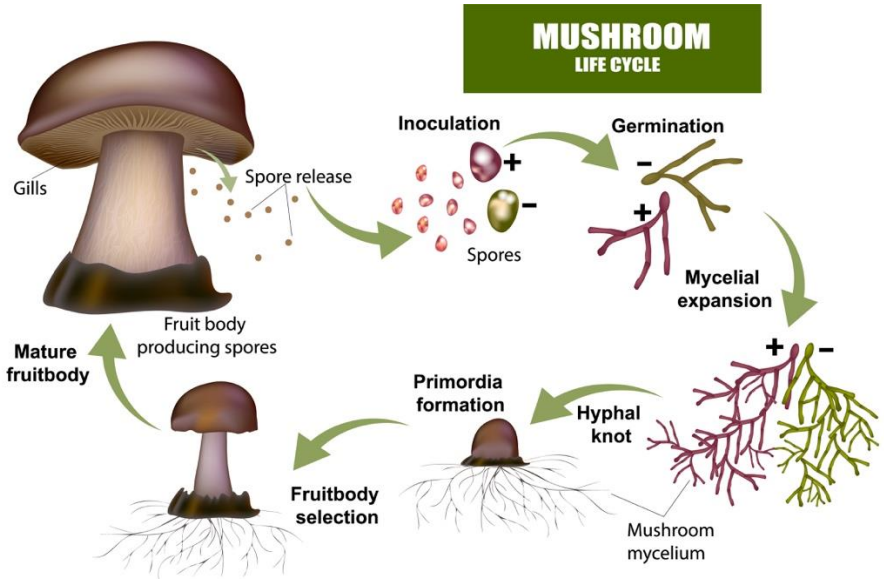


Figure: The life cycle of mushrooms. In some of our kits, we include the mushroom mycelium, which is an important part of a mushroom's life cycle. A full grown mushroom fruitbody will release spores in the environment. Under the right conditions these spores will germinate and expand into what is known as mycelium.

Growing Mushrooms

There are several guides online for growing your own mushrooms. Some of our kits include oyster mushroom mycelium, which grows extremely well on agar plates such as potato dextrose agar or malt extract agar. However, if you want to grow full mushroom fruitbodies, check out some of the videos below:

https://www.youtube.com/watch?v=45b2t7fqhjA

https://www.youtube.com/watch?v=K8DLGsH0XM0

Types of Agar Plates

Microorganisms including bacteria, yeasts, and fungus (including mushrooms) grow very well in the laboratory on petri dishes known as agar plates. Different types of agar plates have different formulas that are suitable for growing different types of microorganisms. Below are three common agar types and their uses:

Nutrient Agar (NA)

Nutrient Agar is a general purpose medium that grows a wide range of microorganisms. It can grow yeasts, bacteria, molds, and other types of fungus. It is an excellent choice for testing microorganisms in the environment and has a wide range of uses. Please check out one of our other E-Books at www.experimentebook.com for several exciting experiments you can do with nutrient agar and other general purpose agars such as tryptic soy agar.

Figure: The above image is a picture of 5 nutrient agar petri dishes from the Evviva Sciences laboratory.

Potato Dextrose Agar (PDA)

Potato Dextrose Agar does not grow bacteria very well but is an excellent medium for growing different types of fungus. It is one of the agar recipes of choice for mushroom growers, and it is also a great choice for testing items for mold.

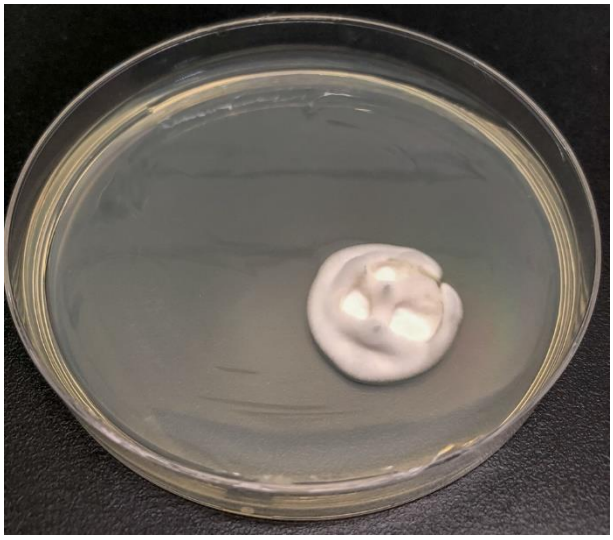


Figure: Growing fungus using potato dextrose agar dishes.

Yeast Peptone Dextrose Agar (YPD)

Yeast peptone dextrose agar is used specifically for yeast cultivation. Some of its ingredients prevent other fungi such as mold from growing and also prevents bacteria from growing. If you want to grow yeast on an agar dish, this formula would be your top choice.

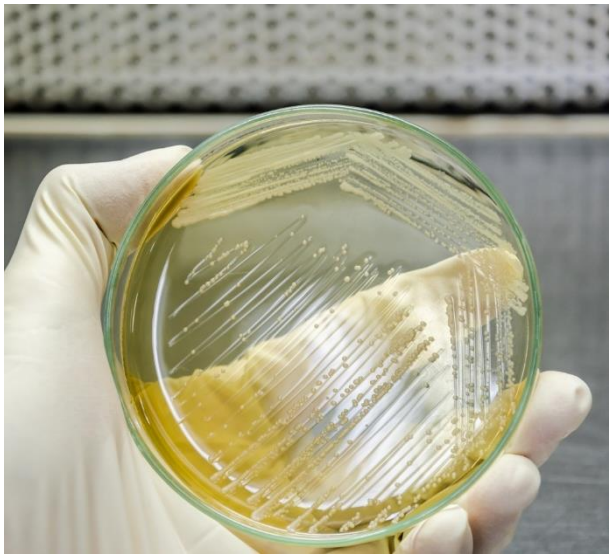


Figure: Using yeast peptone dextrose agar plates to grow Baker's Yeast!

Experiments With Potato Dextrose Agar, Nutrient Agar, & Yeast Peptone Dextrose Agar

The experiments below use our Amazing Agar Variety Kit, which is sold on Amazon.com. If you are interested in trying out this kit, please email us at support@evvivasciences.com, and we will show you where to find it.

Experiment #1: Test for Mold Spores

Materials needed: Potato Dextrose Agar Plates

If we were to measure just the distance our arms can extend, the number of microorganisms can go into the millions! Luckily, people are strong and can fight off nearly every bacteria that tries to invade our bodies. A single cough or sneeze can send millions of germs into the air! This is why doctors wear masks when around sick patients. Those who are sick may have weaker bodies and make it easier for bacteria to attack them. The mask prevents the germs from our mouths to get into the air. For our first experiment we will see these invisible airborne microorganisms for ourselves.

Figure: Testing for mold spores in the air using potato dextrose agar plates.

Step 1: Take a potato dextrose agar plate and simply leave it open on a table or desk for an hour.

Step 2: After an hour has passed, close the lid and leave it in a dark location anywhere from 3 to 7 days. Check the plate every day and see the changes.

Step 3: Soon you will see the mold spores that landed on the plate grow. These are colonies of mold and we are able to see them because the liquid and nutrients on the plate makes it easy for mold to grow.

Experiment #2: Testing for Germs

Figure: Swabbing different items to test for germs using agar plates.

Materials needed: 1 of either the Nutrient agar (NA), potato dextrose agar (PDA), or yeast peptone dextrose agar (YPD) plate.

In this experiment we will test for germs on the physical surfaces around us. Germs are everywhere and are impossible to avoid. Don't worry, similarly to bacteria or mold in the air, our bodies are strong and prevent most of these germs from infecting us.

Step 1: Open one of the sterile cotton-tipped swabs included in your kit. Wet the tip with water as this helps with picking up more microorganisms.

Step 2: Swab different items, such as your phone, the sink, your teeth, or anything you can think of.

Step 3: Add the swab contents to the agar surface of an agar plate. You can use nutrient agar to test for bacteria, potato dextrose agar to test for fungus, and yeast peptone dextrose agar to test for yeasts.

Step 4: After swabbing, close the lid and turn the plate upside down, so that the lid is on the bottom and the agar surface is on the top. (Make sure to record what objects you swabbed for each plate).

Step 5: Check the instruction guide included in your kit to see the optimal incubation temperatures for each different agar type. A rough guideline is that nutrient agar and yeast peptone dextrose plates work well at around 80 to 85deg F. Potato dextrose agar works well at room temperature. Keep the plates at the listed temperatures for optimal microorganism growth. PDA plates work well in the dark, while a desk lamp can be used to heat the NA and YPD plates.

Step 6: Check the progress of the germs and be patient as it may take time to see significant results. Try to guess which will have the most microorganisms!

After some time passes, you will see the bacteria growing on the plate and see just how dirty specific things can be. What was the dirtiest? Were your guesses correct?

Experiment #3: Yeast!

Materials needed: 2 YPD plates, balloon, empty soda bottle

Figure: Experiment #3. The left image demonstrates growing yeast on YPD agar plates, while the right image demonstrates how Baker's yeast can partially inflate a balloon.

When you think of yeast, you probably immediately think about their importance in baking bread. In the next experiment we will see the process of how yeast make bread rise, using a balloon.

Step 1: Fill up a container with 1 cup of water, and heat it until it is lukewarm (~98-110F).

Step 2: Pour a packet of baker's yeast into the water and stir well. Let the mixture sit for at least 10 minutes.

Step 3: Dip a cotton swab into the mixture and spread it across a YPD plate. You can do streaks, dots, or both.

Step 4: Take the 2nd YPD plate and use a cotton swab to break up the agar on a YPD plate. Break it up into small pieces.

Step 5: Put the broken agar pieces into an empty soda bottle. Using a funnel may make this process faster.

Step 6: Once all the pieces are in, add the rest of the mixture you created in step 2 into the soda bottle.

Step 7: Take the balloon from your kit and carefully stretch it out a few times to make inflating easier. After stretching it out, cover the top of the bottle with the balloon. Be careful as the bottle may tip over! After the balloon is on, it helps to lightly shake the bottle.

As the yeast feeds off the agar mix, they will produce carbon dioxide which will inflate the balloon. This inflation is the same process that makes bread rise.

Experiment #4: Mushroom Mycelium

Materials needed: PDA agar plate, oyster mushroom mycelium liquid

Mushroom mycelium is the vegetative part of a mushroom. Mycelium looks like a network of branching white filaments. When mycelium grows on agar, it won't produce full mushrooms. But it is a key part of a mushroom's life cycle.

Figure: Growing mushroom mycelium on an agar petri dish.

Step 1: Add the included liquid oyster mushroom mycelium to a potato dextrose agar plate. You may either dip a cotton swab into the tube and spread/dab it onto the plate, or slowly pour some of the mycelium onto the plate and spread it using a swab.

Step 2: Put the plate in a dark location and check on it daily. Tip: Covering the plate with a box to provide darkness may be a good idea.

After a few days, white fuzzy growth will appear on the plate. While this will not result in the production of a complete mushroom, it is a crucial process for growing them. You may try growing the mycelium on other types of plates to compare the differences in effectiveness between the agar plates.

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