

Identifying an unknown bacterium



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Abstract

A tryptic soy broth containing an unknown bacterium was assigned for identification. Various exercises were utilized to identify the specific features, structures and characteristics of the unknown bacterium. A basic identification procedure of gram staining indicated that the unknown bacterium is gram positive and rod-shaped. Following the gram stain exercise, five tests were performed to extensively learn more of the unknown bacterium's features in depth. The tests that were performed are catalase test, oxidase test, endospore stain, blood agar hemolysis and coagulase test. Catalase test demonstrated the presence of catalase in the unknown bacterium. Catalase is an enzyme that catalyzes the release of oxygen from hydrogen peroxide. After the catalase test, an oxidase test was performed and the results showed that the unknown bacterium does not have the ability to produce the enzyme in the bacteria transport chain called cytochrome c oxidase. The endospore stain determined that the unknown bacterium is an endospore-former, which makes the bacterium able to withstand heat and radiation. When the coagulase test was performed, the results after incubation remained in liquid form and showed no coagulation in the test tube. Lastly, the unknown bacterium was aseptically transferred onto a blood agar plate using the isolation method, and after incubation, the results on the agar showed green-brownish colonies that is described to be an alpha-hemolytic reaction. After the variety of tests, the results were compared to a list of other bacteria's morphologies. The process of elimination technique was used through a flow chart to narrow down the options and eventually identify the unknown bacterium.

Introduction and Definition of a Bacterial Species

Bacterial species are single-celled microorganisms that inhabit different types of environments. In order to properly examine and evaluate a bacterial species, basic laboratory procedures are performed while vital safety precautions are taken. One of the techniques practiced in the laboratory is aseptic transfer technique. Aseptic technique is used to prevent or minimize contamination while handling and transferring microbes onto the appropriate media. Aseptic technique also aids in order to achieve more accurate results for the test while taking safety precautions so it must be practiced at all times. It is very important to wear proper protective equipment such as gloves, laboratory coat and closed-toe shoes while practicing the aseptic technique to prevent exposure to organisms that may be harmful to humans and the environment. Cleaning the lab bench and organizing all appropriate equipment and placing materials within reach, will aid in minimizing contamination and efficiency of the exercise. Organization of materials is very vital in order to correctly test the desired culture, so every media used must be labeled with the correct organism name, student's name, and date. To begin the aseptic transfer from broth to slant culture, the broth culture must be mixed on a vortex mixer to ensure that the organism is well distributed in the broth. An inoculated loop must be sterilized by placing it into a microincinerator approximately for 5-10 seconds. While the broth culture test tube is held with the non-dominant hand, the last two fingers of the dominant hand is used to remove the broth culture test tube's cover, while the dominant hand is still holding the sterilized loop. Only minimal movements must be made while the sterile loop is in hand to prevent

contamination. While the test tube is uncovered, the opening of the broth culture test tube is flamed by slowly passing it in front of the microincinerator, this is done to prevent airborne contamination and create a positive airflow. By flaming the test tube opening, this is creating a positive airflow that will prevent any airborne microorganisms to flow into the tube and microorganisms will flow outwards instead, so the broth culture will stay as pure as possible. The sterile loop is used to obtain a thin film of broth culture from the test tube. While obtaining a thin film of the broth culture, the test tube is carefully pulled away instead of the loop to ensure that the loop would avoid touching the walls of the test tube. The broth culture test tube's opening is to be flamed again and recapped. The slant medium is then opened and the opening is flamed to ensure the same positive airflow. The tip of the loop is placed near the base of the slant and a zigzag pattern towards the opening of the tube is made until the loop is withdrawn from the tube. The slant test tube's opening is to be flamed and recapped. It is vital that the loop is to be sterilized again by replacing it into the microincinerator for 5-10 seconds. The slant is going to be incubated at an assigned time and temperature. After incubation at the assigned amount of time and temperature, a growth may be observed (1).

Microscopic Examination

To further magnify the visual of the growth and observe an individual colony, a compound microscope is used for proper evaluation and examination. The compound microscope has different parts that work together to optimize the best viewing of the specimen. The objective lenses have four various levels of magnification: scanning objective 4x, low power objective 10x, high power <https://assignbuster.com/identifying-an-unknown-bacterium/>

objective 40x and oil immersion objective 100x. Since oil immersion objective lens is the longest objective, it has to be used with oil. Oil causes a light bend between the tip of the objective lens and the specimen on the slide. More detailed information is obtained under oil immersion objective because more light is captured and it has the highest magnification. The ocular lens or the eyepiece is a fixed lens that has 10x magnification and it is where the examiner can view the specimen. The light and coarse and fine adjustment knobs are used so that the resolution may be adjusted until the specimen's details can appear clear and sharp. There are different types of microscopies that may be used in laboratories such as bright-field microscopy, phase-contrast, dark-field microscopy, fluorescence, atomic force microscopy, confocal, electron microscopy, TEM protocol, SEM protocol, and scanning electron microscopy. Each type of microscopy depends on the specimen's morphology and caters to what is to be observed.

The Use of Selective and Differential Media

Using the appropriate type of medium is important in achieving isolation of desired microbial culture. Selective media allow only certain microorganisms to grow and suppress the growth of the other microorganisms. By manipulating the nutrient sources, the microorganisms' environment and the media's components, selectivity can be achieved. MacConkey agar for example, is both selective and differential media that contains lactose, bile salts, neutral red and crystal violet. Its components of bile salts and crystal violet are what make it selective; lactose and neutral red make it differential. On the other hand, differential media is used to allow growth of every microorganism present so identification of between related groups of

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microorganisms may be determined and distinguished. Gram stain is an example of differential media. Gram stain is differential because it divides the microorganisms into gram-positive and gram-negative based on the characteristics of their cell wall. In gram staining, two different types of dyes are used. Each dye color is selectively absorbed by the cell's wall, depending on the cell wall's structure and composition (1).

The Importance of Including Positive and Negative Controls in the Unknown Identification Process

To identify and distinguish the results of a laboratory exercise, it is imperative to obtain both positive and negative control groups. In the identification process of the unknown bacterium, having both positive and negative control will aid in the changes that may occur in both variables. The examiner will be able to make a comparison between the negative control, which is the untreated variable, and the positive control, which is the treated variable. These controls serve as a baseline of the exercise or experiment.

Materials and Methods

Gram stain is one of the differential tests used in determining the cell's group (gram-positive or gram-negative) depending on its cell wall composition. In order to perform a gram stain, a clean glass slide is obtained and labeled with necessary information and marked with a line or an oval to separate the different samples of cultures. Thin smears of desired cultures are aseptically transferred on the slide. The smears of the cultures have to be thin in order for the appropriate stains to penetrate the cell walls and be accurately viewed through a compound microscope. The slide has to air-dry

until there is no visible wetness. When there is no more visible wetness, heat-fixing is done by placing the slide on top of the microincinerator rack for about 20 seconds. This step allows the proteins on the smear to denature and the stains will stick better. After heat-fixing, the smears are covered with crystal violet for one minute. After a minute of crystal violet staining, the slide is held at an angle rinsed with distilled water only at the edges, to ensure that the water does not directly rinse the smears. After rinsing, the smears are covered with iodine for one minute and then rinsed with distilled water the same way with rinsing crystal violet. After rinsing, the slide is very carefully blotted dry with a paper towel. Immediately after blotting the slide dry, the smears are decolorized with 95% ethanol for only 12-15 seconds to avoid over or under decolorizing. After 12-15 seconds, distilled water is again used to rinse and a paper towel to carefully blot the slide dry. After decolorizing, it is important to counterstain the smears with safranin for one minute. The same rinsing and drying methods are done. The slide is ready to be viewed and observed under a compound microscope starting with the scanning objective lens to the oil immersion objective (1). Gram-positive cells contain thick peptidoglycan layer and it retains the primary stain of crystal violet even after it is decolorized by ethanol. Gram-negative cells have a thinner wall layer that consists of lipopolysaccharides that do not retain crystal violet when it is decolorized by ethanol. It is necessary for gram-negative cells to be counterstained with safranin for the gram-negative cells to be visible under bright-field microscopy. Gram-positive and rod-shape are the primary findings on the morphology of the unknown bacterium assigned in this laboratory.

Sources

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