Identification of an Unknown

Enterobacteriaceae

Introduction

This lab experiments serves as an application of all the lessons that were taught and to test if the student understood and remembered the various tests that were mentioned in the laboratory and lecture classes in bacteriology. The purpose of the experimental determination of an unknown was to demonstrate the utility of many tests that indicate the metabolic behavior of the unknown. In this experiment, my unknown #14 was determined to be *Proteus vulgaris*.

Proteus vulgaris is one of the most commonly isolated members of *Proteus* species. The genus *Proteus* is a member of a large gram negative bacilli family, Enterobacteriaceae. Proteus organisms are known to be one of those to cause serious infections in humans, along with *Escherichia, Klebsiella, Enterobacter, and Serratia* species. (Nester et al. 2008)

The bacterium is a gram-negative rod with flagella. As a gram-negative rod, it has an extracytoplasmic outer membrane. It creates an endotoxin, which can cause a deadly systemic inflammatory response in 20 to 50 percent of its victims. It has been shown that its optimal growth temperature was at 37 C. *P. vulgaris* is a chemoheterotroph, which means it uses carbon sources like glucose for energy and carbon. As a chemoheterotroph, it ferments glucose but not lactose or mannitol. However, because it is a facultative anaerobe, the glucose fermentation only occurs in anaerobic conditions. If placed in non-ideal, aerobic conditions, the microbe will use a variety of organic molecules to survive. (Struble, et al., 2009)

When identifying the microbe, several tests can be used. It will test positive on the citrate test and urease test. Because it ferments glucose but not mannitol or lactose, it will only test positive in the glucose tests. Likewise, when observing the plated colonies, it will be noticed on non-selective media a "swarming" behavior, where the microbe grows in waves. The bacterium grows and stops in waves, creating what appear to be distinctive rings. This feature is the result of the microbe's flagella, which allow it to be extremely motile. (Alachi, P. 2007)

Materials & Methods

A broth containing an unknown microbe and labeled with #14 was obtained from the lab instructor. A gram stain was initially performed to determine if the unknown microbe was gram positive or gram negative. If the bacterium was gram positive, it retained the purple color; if it was gram negative, it retained a pink or red color.

MacConkey agar acting as a visual pH indicator, distinguishes those Gram-negative bacteria that can ferment the sugar lactose (Lac+) from those that cannot (Lac-). The test was performed using streak method and incubated at 35 C. If the colonies are pink, they are Gram- lactose-fermenting bacteria. These pink colonies are typically coliform bacteria in the family Enterobacteriaceae, inlcuding the genera *Escherichia, Klebsiella, Enterobacter, Hafnia* and *Citrobacter*. Non-lactose fermenting (yellow), non-coliform members of Enterboacteriaceae include the genera *Proteus, Morganella, Providencia, Edwardsiella, Salmonella* and *Shigella*. (Alachi, P. 2007)

Triple sugar iron agar (TSI) is a differential medium that contains lactose, sucrose, a small amount of glucose (dextrose), ferrous sulfate, and the pH indicator phenol red. It is used to differentiate enterics based on the ability to reduce sulfur and ferment carbohydrates. As with the phenol red fermentation broths, if an organism can ferment any of the three sugars present in the medium, the medium will turn yellow (A/A). If an organism can only ferment dextrose, the small amount of dextrose in the medium is used by the organism within the first ten hours of incubation at 35 C. After that time, the reaction that produced acid reverts in the aerobic areas of the slant, and the medium in those areas turns red, indicating alkaline conditions (K/A). The anaerobic areas of the slant, such as the butt, will not revert to an alkaline state, and they will remain yellow. (Alachi, P. 2007)

Citrate is the test required that a single colony from the plate incubated at 35 ˚C be innoculated on a Simmon's Citrate plate and incubated for 24 hours. Sodium citrate is the only source of carbon in this medium. Bacteria that metabolize citrate into alkaline end products grow while those lacking the enzymes do not grow. A royal blue color change is observed for a positive test; the plate remains green if the microbe is negative for citrate utilization. (Alachi, P. 2007)

Urease test was performed by spot inoculated the unknown on a urea agar plate and incubated overnight at 35 C. The purpose of this test was to check if the enzyme urease is produced to hydrolyzed urea to ammonia and carbon. If urease is produce, then

the pH indicator (phenol red) in the medium detects the alkaline condition from ammonia production and turns the medium bright pink indicating positive result. (Alachi, P. 2007)

Phenylalanine deaminase is an enzyme that degrades amino acid phenylalanine into phenylpyruvic acid which can be detected by adding ferric chloride. First, the unknown is inoculated on a PAD plate. After incubate it overnight at 35 C, a green color on addition of ferric chloride is a positive result. (Alachi, P. 2007)

S.I.M. was to test for production of hydrogen sulfide, indole, and motility. Three test tubes of S.I.M. was then innoculated with the bacteria on the needle stabbed half way through, and incubated for at 35 ˚C for at least 24 hours. The indole test was performed in order to determine whether or not the tryptophan in the broth was converted to indole. Because indole is a component of tryptophan, the broth was converted to indole in the presence of the enzyme tryptophanase, which only some bacteria have. The presence of indole was tested for when the Kovac's reagent was added, which reacts with the indole and produces a red layer at the top of the tube. Sulfide test was used to test for the production of hydrogen sulfide from the domination of the amino acid cysteine, which contains a sulfur-sulfur bond. Some bacteria produce an enzyme called cysteine desulfurase which begins the initial reaction by the sulfur-sulfur bond in cysteine to H_2S. This can only happen if the coenzyme pyridoxyl phosphate is present. A black precipitate will form where microbial growth is occuring; this black precipitate is FeS, where the sulfur and the iron of the deep have reacted. Motility was detected by growth away from the stab line of inoculation. A cloudy diffuse growth is positive for motility. (Alachi, P. 2007)

The oxidase test consisted of testing for the presence of the enzyme cytochrome (indophenol) oxidase. A swab of the culture is taken from the plate and one to two drops of the oxidase reagent (tetramethyl-p-phenylenediamine) was added directly over it. If the bacteria turned black, it indicated that the bacteria were oxidase positive. If there was no color change observed other than the reagent, then the bacteria were oxidase negative. (Alachi, P. 2007)

Table 1-1. Biochemical Test Results for unknown #14

Mac	Citrate	Urea	PAD	TSI	S	I	M
- (yellow)	- (dark blue/green)	+ (pink)	+ (greenish-yellow)	A/A + H2S	+ (black precipitate)	+ (red ring)	+ (cloudy)

When the Gram stain was performed, the unknown appeared to be gram-negative baccili since the microbe was red in color and rod-shaped. After it was determined that the microbe was a gram-negative bacillus, the next step in identifying the microbe was an oxidase test. The oxidase reaction tests for the presence of the oxidative enzyme cytochrome oxidase. In some bacteria, this enzyme is present and can be used to distinguish between two lines of bacteria. During the electron transport chain, the cytochrome oxidase transfers electrons to oxygen; in order to perform this necessary biochemical reaction; cytochrome is oxidized by the enzyme. If the colonies turned purple or black after the reagent was added to a colony, then the test was positive for the enzyme. The colonies would change color, because the redox dye was reduced. In the case of the unknown, the colonies tested negative for oxidase enzyme. (Alachi, P. 2007)

MacConkey test was then carried out. Since the oxidase test was negative, it indicated that the bacteria must have a different means to transport electrons to oxygen. Another method of electron transfer included carbohydrate utilization performing on a MacConkey agar plate. The degradation of the simple sugar, in this case lactose, will produce an acid. Since an indicator was in the plate, the acid produced, if the lactose was metabolized, would turn the plate pink. After incubating, it was examined to find that the broth was still red which indicates that the lactose was not utilized by the bacterium.

Simmon citrate medium showed a positive reaction as evident in the prussian blue color of the agar. The isolate was also found to be positive for urea hydrolysis as shown in the development of a red-violet color in the urea medium. H_2S reaction was seen in TSI butt/slant as demonstrated in the blackening of the medium (A/A H_2S). Gas

production was not seen in TSI. For phenylalanine deaminase test, 4-5 drops of 10% aqueous ferric chloride was added to the slant after which a green color developed indicating a positive result.

S.I.M. showed a motile reaction demonstrated by the brushlike cloudy appearance in the test tube labeled "M". The presence of indole was tested for when the Kovac's reagent was added, which reacts with the indole and produces a red ring at the top of the tube. The unknown tested positive for the production of indole, indicating that the bacterium in question had the enzyme needed to break down tryptophan. The iron peptone tube, after being incubated for 24 hours, had a black precipitate at the top of the deep, where the innoculating needle had first entered. It was at this point where the growth was occurring. It indicated that the unknown microbe was indeed producing H_2S.

These biochemical properties and physical observations helped in the identification of the unknown #14 , which turned out to be *P. vulgaris*.

The tests left several areas for error. First of all, many of the tests needed to be observed after 12-24 hours. When tests were to be observed after a significant amount of time, other biochemical reactions could have occurred thus producing a false answer. For instance, if the lid of the tube was not covered tightly, the potential for false readings on the tests can occur. Or, in the case of the citrate test, the lid of the test tube could have been closed too tightly, thus killing the culture. During the Gram stain, the results can be skewed if the initial culture was too old, thus having either spores or too many dead bacterium; this would have made staining more difficult.

Discussion:

P. vulgaris is an enteric bacterium, which means it is found in the intestinal tract. It is also found in the soil, contaminated water, or decomposing organic substances. While it is a pathogenic bacterium and has been shown to cause urinary tract infections, it is also part of the natural flora of the intestinal tract; and because the microbe can live on

the skin of some people, *P. vulgaris* is a common pathogen in hospital wound infections, especially in the immunosuppressed. (Gonzalez, G. 2004)

Conversely, *Proteus vulgaris* is easily isolated from individuals in long-term care facilities and hospitals and from patients with underlying diseases or compromised immune systems. (Nester et al. 2008)

But, while *P. vulgaris* does contribute to the *Proteus* infections, it is not the most likely candidate for community-bourn disease. However, *P. vulgaris* is the lead pathogen in causing hospital-bourn *Proteus* diseases. Unfortunately, it is not susceptible to ampicillins or cephalosporans. (Nester et al. 2008) The reason the urinary tract is such a hospitable environment for the colonization of the microbe includes the microbe's ability to degrade urea to ammonia with the enzyme urease. (Struble, et al., 2009)

For treatment, the recommended empirical treatment includes an oral quinolone for 3 days or trimethoprim/sulfamethoxazole (TMP/SMZ) for 3 days for uncomplicated UTIs in women on an outpatient basis. Acute uncomplicated pyelonephritis in women can be treated with oral quinolones for 7-14 days, single-dose ceftriaxone or gentamicin followed by TMP/SMZ, or an oral cephalosporin or quinolone for 14 days as outpatient therapy. For hospitalized patients, therapy consists of quinolone, gentamicin (plus ampicillin), or aztreonam until defervescence. Then, an oral quinolone, cephalosporin, or TMP/SMZ for 14 days may be added to complete treatment. (Struble, et al., 2009)

References:

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