Bacteria and their roles in ecology

Bacteria play an important role in the global ecosystem and act as beneficial microorganisms to human beings. Bacteria that do not cause disease in healthy individuals are called normal flora, which instead act as commensalists or mutualists to the host. Even outside of normal flora there are bacteria that produce antibiotics, a commonly used drug to treat bacteria-caused diseases. Bacteria ferments lactose to lactic acid found in dairy products, such as milk and yogurt. Aside from all the benefits that humans get from bacteria, they are widely known as disease-causing microorganisms. Strains of pathogenic bacteria have different levels of malignancy. In order to treat a bacterial infection, the identification and morphology of the pathogen must be known. From microbial classification, we can determine what specific immunologic reagents can be used to treat the disease. The identification of microorganisms begins with isolation of the bacteria and obtaining them on growth media to perform various biochemical tests for accurate species identification. There are many techniques used to determine the morphological and cultural characteristics of various bacteria.

Bacteria from different parts of our body and environment were isolated and inoculated onto primary plates that contain agar-based culture media. A secondary streak plate was used to obtain a pure culture. After a pure culture was obtained, slant agars were used to maintain the bacteria and were newly streaked each week so that they are in an exponential phase growth, which is preferred for the identification tests. Five isolates were taken, each from the anal, skin, forehead, soil spread, and soil environments. The anal culture was collected from the anus. The skin culture was collected

from the back of my wrist. The forehead culture was collected from the area around my nose since it is greasier than my forehead. The soil spread sample was collected in front of the Arts Box Office at UCI, where the soil was moist and in a sunny area. The soil environment sample was collected from a parking lot behind the greenhouse where the soil was in a shaded area beneath the tree and fairly dry. Additionally, an unknown sample #117 was assigned to me. The anal, forehead, soil spread and soil environment samples are tested to identify the genus, whereas the skin sample and unknown sample are tested to identify the microorganisms down to the species level.

Staphylococcus aureus is known to be the predominant bacteria that inhabit the surface of the skin (Todar, n. d.). S. aureus can be isolated from the most superficial layers of the epidermis and the upper parts of the hair follicles. S. aureus is part of the normal flora even though it is a potential pathogen. Bacteria that reside on our skin have to have tolerance against the dryness, high salt concentration, and acids and lipids in order to survive. S. aureus has the ability to grow at temperatures ranging from 15°C to 45°C and at salt concentrations as high as 15 percent (Todar, n. d.).

The first strains of Escherichia coli were isolated from the feces of newborns in 1885, and then it was discovered to inhabit the large intestine as normal flora (Todar, n. d.). E. coli also exists as pathogenic microorganisms, such as those that are diarrhea-inducing. E. coli has a versatile physiology and is well-adapted to different environments. It can use glucose as its only carbon source and grow in the presence or absence of oxygen. In the absence of

oxygen, the bacterium can undergo fermentation or anaerobic respiration. These characteristics that E. coli has allow it to live in our intestinal habitat.

A series of tests, called ABA DABA procedures, were performed to find different characteristics of bacteria to determine its genus and/or species (Woolfolk et al., 2004). Bacteria species fall into two large categories, Gram-negative and Gram-positive, based on their cell wall composition. The ABA DABA charts split the bacteria into these two groups and integrate three different identification tests to identify the isolates to the genus levels. Once we have assigned the genus to the isolates, there are further tests to narrow the identification of the organisms down to the species level. Gram staining was the first test performed to determine which group each of the isolates belonged to. Some of the characteristics that were tested for are the bacteria's ability to ferment glucose and other sugars, to grow aerobically and/or anaerobically, to grow on minimal medium, presence of certain enzymes, flagella, and endospores, carbon and/or nitrogen source utilization tests, requirement for growth factors, tolerance to environmental conditions, and susceptibility to antibiotics.

RESULTS:

According to the ABA DABA chart, the isolate from experiment 2 belongs to the Enterobacteriaceae family because of the test results from the Gram stain, phenol red, motility, and oxidase tests (Woolfolk et al. 67). With further results from the methyl red, Voges-Proskauer, gelatin hydrolysis, lactose fermentation, minimal medium growth, and citrate utilization tests, I am able

to conclude that my isolate belongs to the genus Enterobacter (Woolfolk et al. 82).

The isolate from experiment 3 was determined to be part of the genus Staphylococcus by its cellular morphology along with the Gram stain, catalase, phenol red, and the minimal medium growth tests (Woolfolk et al. 64). Due to the mannitol fermentation (aerobic), coagulase, novobiocin susceptibility, urease production, and trehalose fermentation tests, I am able to conclude that my isolate is the species Staphylococcus aureus (Woolfolk et al. 91).

Due to the observations and results from the isolate's cellular morphology, Gram stain, catalase, phenol red, and the minimal growth tests, the isolate from experiment 4 belongs to the genus Propionibacterium (Woolfolk et al. 64).

The isolate from experiment 6 is from the genus Pseudomonas due to the results from the Gram stain, phenol red, motility, and oxidase tests.

Due to the observation and results from the isolate's cellular morphology, Gram stain, catalase, phenol red, and the minimal growth tests, the isolate from experiment 7 belongs to the genus Bacillus.

The results from the Gram stain, phenol red, motility, and oxidase tests, determined that the unknown isolate belongs to the Enterobacteriaceae family. With further results from the methyl red, Voges-Proskauer, gelatin hydrolysis, lactose fermentation, urease hydrolysis, hydrogen sulfide

production, citrate utilization, and indole production tests, the unknown isolate is found to be from the genus Escherichia.

DISCUSSION:

The anal culture sample was inoculated on Eosin-Methylene Blue (EMB) agar in order to isolate and differentiate enteric intestinal bacteria. The possible isolates that could be present on the EMB agar are lactose-fermenting enteric and non-lactose-fermenting enteric bacteria. Through all the tests mentioned in the results section for experiment 2, the isolate was found to be from the genus Enterobacter, which is a common normal flora in the human gastrointestinal tract. All of the results from the identification tests matched up with the expected results for Enterobacter spp., except for citrate utilization where the expected result was positive. The citrate utilization test was only done once, so it is possible that there was improper inoculation onto the citrate agar slant. The only provided organism that has a negative result from the citrate test is from the genus Erwinia (Amylovora group), but the tests for this organism contains many results that are opposite that of the isolate from experiment 2 (Woolfolk et al. 82).

The skin culture sample was inoculated on mannitol salt agar, which contains 7. 5% NaCl making it selective for microorganisms that can grow in high salt concentrations. The isolate was expected to be a microorganism that is salt-tolerant and able to ferment mannitol. The most common and expected bacteria to be isolated from the skin are from the genera Staphylococcus, Streptococcus, Corynebacterium, Propionibacterium, and/or Mycobacterium (Woolfolk et al., 2004). These bacteria are known to be part of our normal

flora. The species identification test results matched up to the expected results nicely for S. aureus (Woolfolk et al. 91). The results all came out to be positive tests and this eliminated the other possible commonly isolated species of the genus Staphylococcus. The production of coagulase was important in determining that the isolate was S. aureus and not any of the other given species. The focus on this bacterium has become important since there has been an increase of methicillin-resistant S. aureus (MRSA), especially in hospitals. MRSA strains are shown to be resistant to multiple antimicrobial agents. Their ability to spread rapidly in different environments suggests that MRSA strains contain unique virulent factors and efficient mechanisms of pathogenesis (Lowy, 1998). S. aureus is commensal as it resides on the skin, conjunctiva, nose, pharynx, mouth, lower gastrointestinal tract, anturethra, and vagina (Todar, n. d.). Infection by S. aureus occurs when there is a damage of the skin or mucosal barrier that will allow the Staphylococcus to have access to adjoining tissues or the bloodstream (Lowy, 1998).

The forehead culture was inoculated onto a sodium lactate plate, which contains NaCl and sodium lactate to select for growth of bacteria that can grow in a high salt environment. This culture was grown and maintained in an anaerobic condition to see growth of anaerobic bacteria. The collected sample was from a greasy area on my face. The expected bacterium that should have been isolated is the genus Propionibacterium. Propionibacterium is an anaerobic bacterium that inhabits in the deeper layer of our skin in the sebaceous glands. Since Propionibacterium inhabits beneath the layer of oil on our skin, it should be able to grow anaerobically since its environment

contains very little to no oxygen. I would re-do the ABA DABA tests, especially the anaerobe test, because there was about the same amount of growth when the bacteria was grown in aerobic conditions when compared to the one grown in anaerobic conditions. If the bacteria have more growth abundantly in aerobic conditions, then the genus would be Corynebacterium.

The expected soil sample spread organism to be isolated from S1 agar is fluorescent Pseudomonas, which matches with the isolate. The isolate produced a green-yellow pigment on the S1 agar.

The soil environment expected species to be isolated from nutrient agar is the Bacillus species, an endospore former. The common genera of bacteria that can form endospores are Bacillus and Clostridium. One of the differences between the two is that Bacillus spp. is aerobic or a facultative anaerobe and Clostridium spp. is anaerobic. The isolate was grown in the presence of oxygen; therefore it could not have been part of the genus Clostridium.

For the unknown species the ABA DABA tests showed that the isolate was -ABA, which is the 3-letter code assigned for the family Enterobacteriaceae. To further test for the genus, more biochemical tests were required. However, -ABA isolates could not be identified to the species level due to the high number of tests that was required. From the additional tests that were performed on the unknown isolate, I was able to narrow the species of the unknown isolate down to two given species. From the characteristics and properties that the isolate has, it is most likely to be the species Escherichia coli. The other species that this unknown isolate can be is Escherichia

adecarboxylata. There are several other tests that would need to be performed in order to confirm that the isolate is E. coli. The additional tests that would distinguish E. coli from E. adecarboxylata include testing for the ability for the organism to synthesize lysine decarboxylase and/or ornithine decarboxylase, growth in KCN, D-Adonitol and/or D-Arabitol fermentation, and being able to use cellobiose as a carbon source (Woolfolk et al. 83). E. coli is an important bacterium that needs our focus and attention due to its major role in diarrheal diseases, especially in the developing world. Strains of enterotoxigenic E. coli (ETEC) are known to be a cause of cholera (Sack, 1980). ETEC are strains of E. coli that has the ability to produce enterotoxins that cause a secretory response. Common exposure to this strain of E. coli is through fecal contamination of water and food. E. coli is part of fecal normal flora, but outside of its common niche it is an opportunistic pathogen. ETEC produces two enterotoxins, heat-labile (LT) and heat-stable (ST), whose activities are controlled by the DNA in transferrable plasmids. LT has a similar function to the enterotoxin of Vibrio cholera, the cause of cholera in humans (Sack, 1980).

The purpose of this lab was to perform various relevant biochemical tests to narrow down the identification of the microorganisms isolated from different environments. After understanding the basic concepts of how these biochemical techniques work, we can go on and further identify specific strains from the known species. Like many other bacteria, S. aureus and E. coli have non-pathogenic and pathogenic strains. By studying the differences between non-pathogenic and pathogenic strains, we can compare their different structures and components and possibly develop antimicrobial

agents to fight against the disease-causing bacteria and prevent their widespread.