the comparative analysis of simple staining essay sample

INTRODUCTION:

A German bacteriologist, Dr. Theodore von Escherich, was the first man in 1885 who discovered the bacterium named Escherichia coli, which are gram negative and appears in rod shaped. Most kind of bacteria E. Coli does not cause diseases and some strains indeed are beneficial in helping the process of food breaking down in the intestines. However, “ the most infamous strain E. Coli O157: H7”, which caused the outbreak of Jack in The Box hamburger in 1993 and the recent spinach outbreak in 2006, spread out abdominal pain and diarrhea on the civilization (American et al., 2011).

Streptococcus pyogenes is a spherical, gram-positive bacterium, which was discovered by Hippocrates, who was known as “ Father of Medicine” in the fifth century B. C. (Leyro et al, 2008). Streptococcus pyogenes affects its hosts in many different ways and causes large ranges of diseases, which includes both mild and serve disease, such as such as fever, severe pain, dizziness, and red rash. S. Pyogenes can destroy both red blood cells and white blood cells, which is responsible for being immunization (Todar et al, 2012).

Both of bacteria, E. Coli and S. Pyogenes, were observed under the compound light microscope, which is a type of microscope using visible light and a system of lenses to magnify the images. In this second lab experiment, four slides of bacteria were viewed under the compound light microscope from 40X up to 400X total magnification for the purpose of giving the images of bacteria in detail beautifully.

Moreover, the most important part of the experiment was the studying of being familiar to the two primary staining techniques, which were simple staining and gram staining. In the simple staining technique, the chemical methylene blue helped penetrating the cell wall and allowed the cell to be visible. Last but not least, the second staining technique, the gram staining, was beneficial on separating the bacteria into gram positive and gram negative under the compound light microscope. Especially, the technique of heat fixing killed the organisms without serious distortion, so their adherence would improve to stick on the slide and also took up dye more easily.

In this study of the lab experiment, the improvement of the knowledge of using simple staining and gram staining is beneficial in the study of microbiology in the future. The ability of distinguishing the difference between gram positive and gram negative bacteria by using gram staining technique also serves as a potential object to understand and discover more about all the bacteria in the world.

MATERIAL AND METHODS:

Materials: The compound light microscope, bibulous paper, methylene blue, crystal violet, iodine solution, ethanol solution, safranin, E. Coli, and S. Pyogenes samples were provided by the biology department of Texas Southern University.

Methods: The E. Coli and S. Pyogenes were scratched on each two slides and heat-fixed on the Bunsen burner. In simple staining procedure, two slides of E. Coli and S. Pyogenes were covered with methylene blue, rinsed with water, and observed under the compound light microscope up to 400X total

magnification. In gram staining procedure, the remained two slides of E. Coli and S. Pyogenes were covered with crystal violet in one minute then washed off with water gently. Obtaining iodine solution to cover two slides for another one minutes the rinsing with water again. Using ethanol solution to decolorizing the bacteria in the slides then rinsing with water immediately. Lastly, the safranin stain was applied on two slides for another one minute before rinsing with water. After drying gently the two slides with bibulous paper, the E. Coli, and S. Pyogenes samples were observed under the compound light microscope with magnification up to 400X.

RESULTS:

Figure 1: The sample of E. Coli slide was observed under 400X magnification of the compound light microscope in simple staining method. The E. Coli as seen was in the rod shape and in blue color because the electropositive charge penetrated the cell wall and stained E. Coli in blue. The E. Coli also had the arrangement in several cluster groups, which made the different in image size of the blue dot in the picture.

Figure 2: The sample of S. Pyogenes slide was observed under 400X magnification of the compound light microscope in simple staining method. The S. Pyogenes as seen was in the spherical shape and as similar as E. Coli, the electropositive charge penetrated the cell wall and stained S. Pyogenes in blue. The image of S. Pyogenes was absent in their cluster arrangement and morphology.

Figure 3: The sample of E. Coli slide was observed under 400X magnification of the compound light microscope in gram staining method. The E. Coli as

seen was in the rod shape and in pink color. The E. Coli, which is the gram-negative bacterium, has a cytoplasmic lipid membrane, a peptidoglycan layer, and a lipopolysaccharide layer that absorbs the counterstain safranin to decolorize and give a pink color.

Figure 4: The sample of S. Pyogenes slide was observed under 400X magnification of the compound light microscope in gram staining method. The S. Pyogenes as seen was in the spherical shape and in slightly reddish purplish color. The S. Pyogenes, which is gram-positive bacterium, has a cell wall containing higher peptidoglycan and lower lipid than gram-negative bacteria. Therefore, the diffusion of crystal violet and iodine retains on the cell wall and cannot be decolorized by ethanol.

DISSCUSION:

The conclusion describes that all the procedures in the experiment being conducted properly and successfully. In the simple staining method, the electropositive charge penetrated the cell walls and stained both E. Coli and S. Pyogenes in blue. In slide one, the E. Coli had the rod shape in blue and the arrangement in clusters but the amount of bacteria appeared not too much comparing to the expectation. The assumption is that the E. Coli on the slide was too little to be observed under the compound light microscope. In slide two, the S. Pyogenes was penetrated by the electropositive charge and the cell wall was stained in blue color.

The S. Pyogenes was successfully appeared in the spherical shape but the arrangement of the bacteria was disappointed. The morphology and arrangement of the S. Pyogenes was totally absented in the image,

comparing to the researched sample image on the Internet. The reason assumingly can be either the occurrence of the mistake in starching the amount of bacteria on the slide or the imperfection of the heat-fixing technique, which had caused the S. Pyogenes not to accept the methylene blue easily.

The gram staining method with E. Coli and S. Pyogenes was conducted more successfully than the simple staining method. In the gram staining method with slide one, the cell wall of the E. Coli, which is gram-negative bacterium, contains less peptidoglycan and higher lipid, which causes that the diffusion of crystal violet and iodine easily to be decolorized by ethanol then absorbs the counterstain safranin, and appears in pink color. In slide two of the gram staining method, the cell wall of the S. Pyogenes, which is gram-positive bacterium, appeared in slightly reddish purplish color because the cell membrane had more peptidoglycan and lower lipid than gram-negative bacteria; therefore, causing the diffusion of iodine and crystal violet to retain on the cell membrane despite the decolorization of ethanol.

After the observation on the bacteria under the compound light microscope, the dissatisfaction was raised because most of the important organelles, such as mitochondria, nucleus, and ribosome were not appeared due to the lack of resolving power and total magnification of the compound light microscope. In conclusion, the experiment of using the simple staining and gram staining techniques improves the skill at observation the morphology and structure of bacteria and the ability of distinguishing the difference between gram positive and gram negative for the purpose of understanding and discovering more knowledge about the bacterial world.

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