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Identification of bacterial morphology and catalase coagulation test on *propionibacterium acnes* bacteria

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ABSTRACT

Infection is a way for microorganisms such as bacteria, viruses and fungi to enter the skin which can cause skin problems such as acne. purpose of this study was to determine the morphology of p. acnes by gram staining method, then the catalase test was used to determine the ability of microorganisms to decompose hydrogen peroxide by producing catalase enzymes and coagulase test is a bacterial examination to detect the formation of coagulase enzymes that bind to the bacterial cell wall. The results obtained were that the morphology of the bacteria was in the form of salt and purple in color, the catalase test was positive and the coagulase test was positive. The conclusion obtained was that P. acnes ATCC 11827 was included in the group of gram-positive bacteria based on the results of gram staining and catalase and coagulase tests.

1. Introduction

Infection is the entry point for microorganisms such as bacteria, viruses, and fungi into the skin which can cause skin problems such as acne [1]. Acne or acne vulgaris is infection or inflammation that occurs in many parts of the body produce oil like facial skin. Basically acne is caused by excess oil activation, causing clogged pores or ducts of oil glands on facial skin and hair (pilosebaceous ducts) [2]. Acne can also be caused by a bacterial infection. As for The most dominant bacteria can trigger the formation of pimples namely Propionibacterium acnes [3]. *P. acnes* bacteria is a pathogenic bacteria that belongs to anaerobic Gram positive group. *P. acnes* bacteria act in the formation of pimples on the

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skin that work in producing lipase with break down free fatty acids from skin fat so as to trigger the occurrence inflammation of the skin [4].

P. acnes bacteria is a gram-positive bacteria that belongs to the flora normal on the skin [5]. *P. acnes* bacteria are rod-shaped, no spore-forming, and anaerobic [6]. These bacteria produce lipase which is broken down into triglycerides, one of the components of which is sebum broken down into free fatty acids. *P. acnes* [7]. *P. acnes* is a normal flora that exists in several parts of the body man. This bacterium has been around since infancy with small and increasing amounts many times entering the age of puberty associated with increased production sebum in the sebaceous follicles. The skin is the main habitat of *P. acnes*, however can also be found in the oral cavity, large intestine, conjunctiva and ducts outer ear [8]. Identification of the morphology of the *P.acnes* bacteria is important to know in order to provide information on the properties of the characteristics possessed by the bacteria and to provide the characteristics of the bacteria based on the results of the catalase and coagulase tests The purpose of this study was to determine the morphology of p. acnes by gram staining method, then the catalase test was used to determine the ability of microorganisms to decompose hydrogen peroxide by producing catalase enzymes and coagulase test is a bacterial examination to detect the formation of coagulase enzymes that bind to the bacterial cell wall.

2. Method

This research is included in the experimental research by identifying the morphology of the bacteria and testing the physical properties by testing the bacteria on the catalase and coagulase tests. The sample used was a colony of *P. acnes* bacteria. identification of bacteria p. acnes is started with morphological testing, catalase and coagulase tests. The way of working in this research is as follows.

2.1. Sterilization of tools and materials

Equipment and materials used in the sterilization test first. Tools such as beakers (*Iwaki*), tubes reaction (*Iwaki*), stir bar (*Iwaki*), Erlenmeyer (*Iwaki*), petri dish (*Iwaki*), or other such test materials NA (*Himedia*) and MHA (*Himedia*) media and distilled water were sterilized using an autoclave at room temperature 121 ºC for 15 minutes. Bacterial colonies were obtained from the parent bacteria *P. acnes* ATCC 11827 were obtained from the Microbiology Laboratory, University of Indonesia. For tools such as wire loops, the spatula is sterilized with by heating it over a flame [9].

2.2. Identification of bacterial morphology by gram staining

One Bacterial loops were taken from the NA medium and placed on a glass slide, widened and bacterial fixation was carried out. Next, pour 3 violet crystals drops, let stand for 5 minutes. Then washed with running water, added lugol and let stand for 60 seconds then wash again with running water. Then immersed in a vessel containing acetone alcohol while shaking for 30 seconds. Then wash again with running water, then stained with 3 drops of safranin, let stand for 1 minute, and washed under running water and then dried. The final stage of preparation dried and observed using a microscope with a magnification of 100 x [5]. Bacteria identified when Gram positive bacteria are colored purple and Gram negative in red. The size and shape of the bacterial cell Observations were made, whether round (coccus), rod (bacilli), or wavy (spiral) [10].

2.3. Catalase test

Suspension of P. acnes bacteria was inoculated inside test tube containing 6 mL of NA medium, then dripped with hydrogen peroxide (H2O2) 3 drops using a micropipette. If there are air bubbles indicates that the reaction is positive and if there are no bubbles air in the test tube, the reaction is negative [10].

2.4. Coagulase test

The coagulase test was carried out using the test tube method, used to determine the presence of free coagulase by means of 6 mL of plasma Rabbits were put aseptically into sterile test tubes. A total of 3 The tested *P. acnes* culture colonies were added to the test tube later mixed carefully. Next, the tube is inserted into the incubator on temperature 37^{o}C. Observations were made in the first 4 hours, and after 18-24 hours. A positive reaction will occur if a clot or jelly is formed and when the tube is tilted jelly remains at the bottom of the tube [11]. The use of test animals has obtained permission for ethical eligibility from the ethical commission of the medical faculty of the gastric mangkura university with number 894/KEPK ULM/EC/XI/2021.

3. Results and Discussion

3.1. Identification of bacterial morphology by gram staining

Staining in bacteria aims to show the differences between cells bacteria or bacterial cell parts. Based on response to staining Gram, bacteria are divided into two kinds, namely Gram positive and Gram negative bacteria. The difference between these two bacteria is from the structure the cell wall. This makes Gram positive bacteria produce color purple compared to Gram negative bacteria will produce a red color young if Gram staining is done [12]. Identification Results Bacterial morphology and Gram stain can be seen in Figure 1.

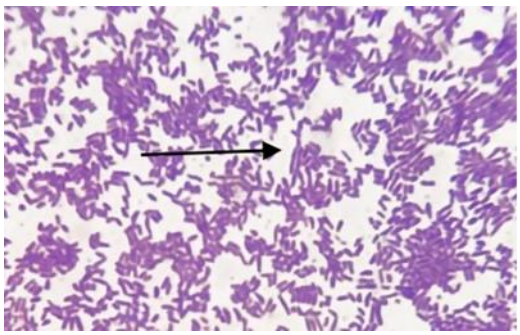

Figure 1. Morphology of *P. acnes* bacteria ATCC 11827

The results of the morphological test and Gram staining showed that The morphology of the *P. acnes* bacteria shows the characteristics of a non-stick shape arranged and shown on Gram stain shows purple colored bacteria which indicates that this bacterium belongs to the Gram positive group. These results are in agreement research conducted by [6] which stated that the bacteria *P. acnes* is rod-shaped and belongs to the type of Gram-positive bacteria [5]. Gram positive bacteria have a purple color when observed with using a microscope, was able to retain the crystal violet dye on during Gram staining [1]. Gram positive bacteria after Gram staining process will produce a purple color when observed under the microscope. This is due to the cell wall of Gram positive bacteria composed of

peptidoglycan which is thicker than Gram negative bacteria. The thicker petidoglycan is able to retain the crystal violet dye. According to [7], there is a phosphoric ester element in Gram bacteria positive because it has a cell wall consisting of two layers viz thick peptidoglycan and inner membrane. This peptidoglycan layer can bind crystal violet dye. Gram positive bacteria appear in color purple due to ribonucleic acids in the cytoplasm, Gram positive cells

3.2. Catalase test

Catalase test is used to determine the ability of microorganisms to break down hydrogen peroxide to produce the enzyme catalase. A positive reaction is indicated by the presence of air bubbles if there are no air bubbles in the test tube then the reaction is negative [13]. The results of the catalase test can be seen in Figure 2.

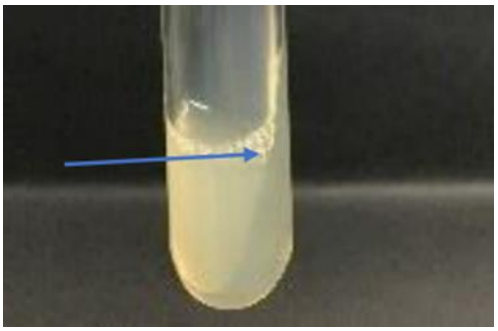

Figure 2. Results of the catalase test for *P. acnes* bacteria ATCC 11827

The catalase test on P. acnes showed positive results. Based on the results obtained are in accordance with the research of [2] which states that P. acnes showed positive result on catalase test, which means Bacteria form catalase which is indicated by the emergence of air bubbles. In [1], also stated that in the catalase test, positive P. acnes bacteria which is indicated by the formation of bubbles air after the addition of H2O2. There are bubbles due to bacteria have catalase enzymes that can break down H2O2 into H2O and O2 [7]. Hydrogen peroxide is a poison that can be damaging bacterial metabolic system. Bacteria will experience death if you can't breaks down hydrogen peroxide into other harmless compounds, This breakdown can be done if there is a catalase enzyme [10].

3.3. Coagulase test

The coagulase test was carried out using the test tube method, a positive reaction would be Occurs when a clot or jelly forms and when the tube is tilted the jelly remains at the bottom of the tube [4]. The results of the coagulase test can be seen in Figure 3.

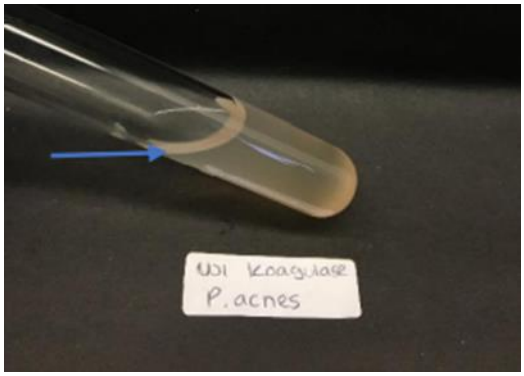

Figure 3. Results of the coagulase test for *P.acnes* bacteria ATCC 11827

The coagulase test is a bacterial examination for detection the formation of coagulase enzymes that bind to the bacterial cell wall [8]. Coagulase test on *P. acnes* bacteria showed positive results but the coagulation power was not great. These results are in agreement research conducted by [13] which stated that the test coagulase on *P. acnes* showed positive results, but power coagulation is not as big as *Staphylococcus aureus*. Coagulase is a protein extracellular substance that can agglutinate plasma with the help of factor yang present in serum. Therefore the role of the resulting coagulase can used as a diagnostic tool [13]. *P. acnes* bacteria provide positive result due to being able to change the reactive coagulase factor, plasma citrate or plasma EDTA in serum [13]. This factor reacts with coagulase and generate esterase and clotting activity in a manner that the same, namely the activation of prothrombin to thrombin [10]. The coagulase enzyme reacts to form a cleavage complex fibrinogen in plasma and causes the formation of fibrin clots in surface of P. acnes, which is able to protect the bacteria from consequential cell damage cell phagocytic action [1]. The function of the coagulase enzyme in *P.acnes* to determine the ability of the microorganism to produce coagulase enzymes. Bacteria will detect the formation of coagulase enzymes bound to the bacterial cell wall [13].

4. Conclusion

The conclusion in this study was that the morphology of the *P. Acnes* ATCC 11827 bacteria showed characteristics, namely irregular rod-shaped and visible on Gram staining showing purple colored bacteria which indicated that these bacteria belonged to the Gram positive group. a positive result on the catalase test, which means the bacteria form catalase which is indicated by the appearance of bubbles. The coagulase test on *P. acnes* bacteria ATCC 11827 showed positive results but the coagulation power was not great.

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