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تطوير استخدام نترات الفضة في تصبيغ البكتريا

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الخلاصة :

طورت طريقة للكشف عن البكتريا باستخدام محلول السلفر نتريت بتركيز ٢% وبشكل خاص لأنواع البكتريا المكونه للسبورات(الابواغ) والكبسولة. ذلك بإضافة المحلول المحضر لنموذج البكتريا المثبته على الشريحة وتركها لمدة عشرة دقائق (*Mycobacterium tuberculosis*, *Bacillus* sp. *Klebsiella* sp. bacteria formation capsule, and *Staphylococcus aureus*). ومن ثم تعريض الشريحة الى الاشعة الفوق البنفسجية لمدة ٢٥ دقيقة حيث يلاحظ تغير لون نموذج البكتريا المثبته على الشريحة الى اللون الوردي- بني فاتح نتيجة اختزال معدن الفضة واختراق الفضة للخلية وبذلك تظهر الخلية البكترية بشكل مضيء تحت المجهر الضوئي مما يميز الخلية البكترية.

الكلمات الدالة: نترات الفضة، صبغة البكتريا، اساس عمل نترات الفضة، طرق استخدام نترات الفضة.

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Developed use of silver nitrate in staining of bacteria

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Abstract

It has developed a method for the detection of bacteria using solution of Silver nitrate in concentration of 2%, particular to spore forming and capsulated types of bacteria. The bacteria were fixed on the slid, and adding prepared silver nitrate solution to stain bacteria cells such as (*Mycobacterium tuberculosis*, *Bacillus* sp. *Klebsiella* sp. bacteria formation capsule, and *Staphylococcus aureus*) on the slide and left for ten minutes at room temperature. Later, exposing the slid to ultraviolet rays for 25 minutes where the color change of fixed bacteria sample on the slide to red - light brown color as a result of the reduction of silver metal which penetrate the cell. As result brighten bacterial cells were examine under an optical microscope, which distinguishes the bacterial cells.

Keywords:Silver nitrate, Stain of bacteria, Silver nitrate for stain agarose, Silver nitrate staining principle, Silver nitrate staining protocol.

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1-Introduction

Silver staining was first presented as a tool for visualization of proteins in 2-DE gels in 1979. Silver staining is one of the most sensitive protein-staining method and can detect proteins down to the Nano gram level [1]. A silver staining method used routinely for detecting bacterial lipopolysaccharide (LPS) in sodium dodecyl sulfate-polyacrylamide gels [2].

The bacterial cell whether it is a coccus or a bacillus will have some structures common. These structures are cell wall, cell membrane, cytoplasm, ribosomes and the chromosome. Other intra-cellular structures such as plasmid, inclusion bodies and extra-cellular structures such as capsule, fimbriae and flagella are possessed only by some bacteria [3].

Silver staining is one of the commonly used procedure for visualizing proteins in acrylamide gels. All silver staining methods rely on the reduction of ionic to metallic silver to provide metallic silver images; the selective reduction at gel sites occupied by proteins compared to non-protein sites is dependent on differences in the oxidation-reduction potentials at these sites [4].The Von Kossastain method (Calcium Stain) is intended for use in the histological visualization of calcium deposits in paraffin or frozen sections. This method is

not specific for calcium itself but tissues are treated with a silver nitrate solution and the silver is deposited by replacing the calcium reduced by the strong light, and so can be visualized as metallic silver[5].

2-Material and Methods

The silver nitrate solution (ALPHA Chemika Compony, India) (2%) was prepared by adding two grams of silver nitrate in 100ml of distilled water. Prepared bacteria membrane by fixed the bacteria on the slide as (*Mycobacterium tuberculosis* (the treatment of tuberculosis center-Erbil), *Bacillus* sp. *Klebsiella* sp. bacteria formation capsule, and *Staphylococcus aureus*) from (Biology department –Science collage- Salahaddin University), cover the slide with silver stain solution by dropper, dry the slides under room temperature, then expose the slides to UV light for 20min (hood with UV light in Biology department –Science collage- Salahaddin University), finally washed the slides by distilled water and exam under light microscope (100X).

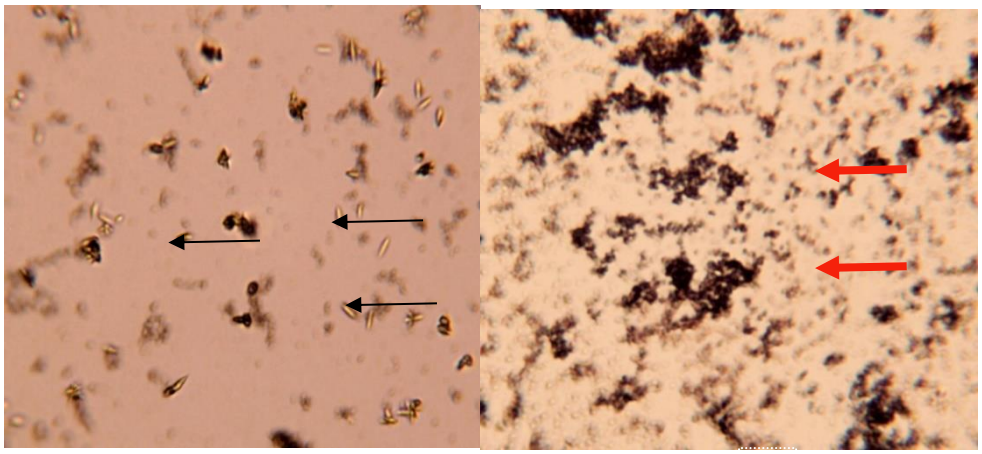
3-Results and Discussion

In this study stain the bacteria by 2% silver nitrate solution, as ‹*Mycobacterium tuberculosis* spore formal bacteria, and *Bacillus* sp. (Figure 1), and *Klebsiella*capsule formation bacteria and *Staphylococcus aureus*gram positive bacteria (Figure 2).

This technique is particularly useful for examining ridges near their anterior termination at the base of the cephalic expansion and for patterns in the cervical zone of nematode. The entire synlophe. is stained, promoting detailed examination ofthe posteriad extent of the ridges in males and females Characters may be revealed with this method that are otherwise difficult to observe using

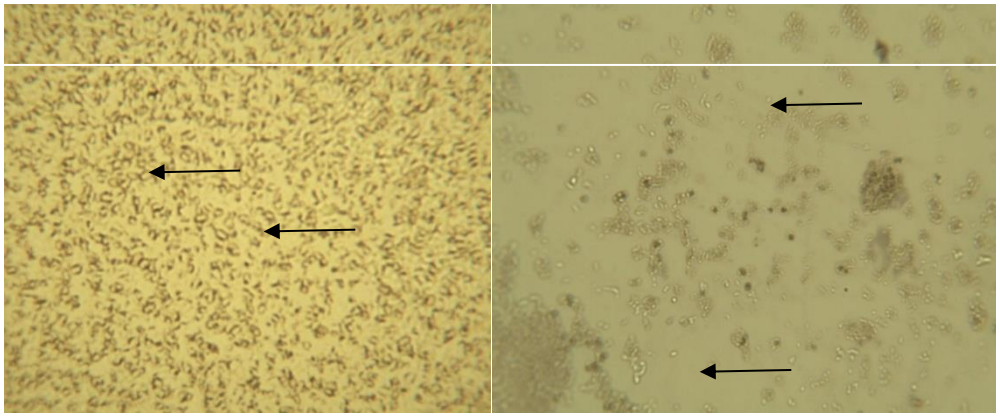
either standard or interference-contrast Light microscopy and as such will substantially augment the study ofthe synlophe in entire specimens as currently practiced [6].

In protein detection used classical Coomassie Brilliant Blue staining can usually detect a 50 ng protein band, while silver staining increases the sensitivity typically 50 times [7]. Could use silver nitrate instate of other stain may be could not get it or expensive, or some times the normal stain need more time.



A B

Figure 1: Bacterial staining by silver nitrate, A. *Mycobacterium tuberculosis*, B. *Bacillus* sp.



A B

Figure 2: Bacterial staining by silver nitrate, A. *Klebsiella*sp. bacteria formation capsule, B. *Staphylococcus aureus*gram positive bacteria.

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