Acta Medica Okayama

Volume 16, *Issue* 1 1962 *Article* 5

FEBRUARY 1962

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Abstract

A selective and simultaneous staining method for nuclear apparatus and cytoplasmic membrane of some bacteria has been presented. Nuclear apparatus is stained with basic fuchsin after hydrolysis with I N HCl and cytoplasmic membrane is restained with Victoria blue 4R after treating with saturated mercuric chloride. By this method, the nuclear apparatuses of B. subtilis, Sal. typhi 57 and Staph. aureus were stained red, and the cytoplasmic membrane and septum bluish purple distinctly. Thus this staining method would be of a great advantage in displaying the cellular structures of the bacteria.

*PMID: 14030606 [PubMed - indexed for MEDLINE]

Acta Med. Okayama **16**, 33—36 (1962)

SELECTIVE STAINING OF CYTOPLASMIC MEMBRANE AND NUCLEAR APPARATUS OF BACTERIA

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Received for publication, January 10, 1962

The submicroscopic structure of bacterial cell has been revealed in detail with the development of the technique in electron microscopy. And cytochemical observations are contributing in revealing the chemical constituents of each part of bacteria, but we have not any satisfactory methods to stain the nuclear apparatus and cell membrane selectively which is required first to distinguish bacterial strain under light microscope.

For this purpose attempts were made by some investigators. GUSTAIN[1] reported a method to stain the nuclear apparatus of *Staphylococcus*, *Pneumococcus*, mould, etc. Besides, the simultaneous staining method of both of nuclear apparatus and cell wall was successfully achieved by CASSEL[2], and this staining method is generally used because of its usefullness in studying the morphologic structure of bacteria. ROVINOW[3] attempted to stain selectively the cytoplasmic membrane of *Bacillus megatherium* by some decoloration procedure after the nuclear staining.

So, the author tried to stain the cytoplasmic membrane and nuclear apparatus of bacterial cells simultaneously but more distinctly. In this paper the results obtained by staining a few strains of bacteria with basic fuchsin and Victoria blue 4R are demonstrated.

MATERIALS AND METHODS

B. subtilis, *Sal. typhi 57* and *Staph. aureus* in stock served as materials. These organisms were grown on nutrient agar plate. After 12-hour culture a small agar block, on which the bacteria grew forming a thin film, was cut off 5 mm. in size placed on a glass slide, cells up, and then kept in a closed vessel. These were exposed to osmic tetraoxide vapor for 2 min. at room temperature by placing a few drops of 2 per cent OsO_4 solution into the vessel. This fixed bacterial cell film was then imprinted on a slide glass to make a fixed cell film. The fixed films were well dried up. Then the films were hydrolyzed in 1 *N* HCl at 60° C for 7 min. and washed with water gently. The films were

treated with saturated mercuric chloride solution for 5 min. and stained again for 30 sec. in 0.05 per cent Victoria blue 4R. The films were gently washed in water, mounted in water with a cover slide and examined under an oil immersion lens.

OBSERVATION AND DISCUSSION

On the specimens stained with the above-mentioned method, nuclear apparatus appeared red being stained by basic fuchsin and cytoplasmic membrane purple by Victoria blue 4R in all strains of the bacteria examined. Observations reconfirmed the fact described by ROBINOW and others[3] that nuclear apparatuses were stained by basic dyes. Generally, the bacteria are stained red homogeneously by basic fuchsin. Bacterial cells contain a quantity of DNA and RNA by which the cells are stained uniformly by basic dyes making the localization of nuclear apparatuses indistinguisable. The result shows that RNA should be removed to see the nuclear apparatus distinctly by staining with basic dyes. Hydrolyzation by 1 *N* HCl for 7 minutes at 60° C was enough to remove RNA from the cells as reported by PIEKARSKI[4]. The author obtained satisfactory results by this hydrolysis on all organisms used in this study. Hydrolysis by chilled perchloric acid, Cassel's method, was also studied and results were as good as with HCl.

Cytoplasmic membrane of bacteria can be stained by Victoria blue 4R as reported by ROVINOW and MURRAY[3]. This dye belonging to those of phenolmethane group has some affinity to the lipids, probably the phospholipids and stains cytoplasmic membrane, but not the cell wall. But with the lapse of time the dye arrested at the cytoplasmic membrane diffuses into the cytoplasm making the picture of the membrane ambiguous. Pretreatment with saturated mercuric chloride solution can appreciably slow down the diffusion of the dye. Water mounting offers an advantage in this process because of the rapidity in observation.

Photo 1 shows the picture of *B. subtilis* which has been stained by the method mentioned, and two to four nuclear apparatuses can be distinguished in a cell. Also the migration process of nuclear apparatus in division can clearly be recognized. The cytoplasmic membrane is stained bluish purple. The transverse septum appearing on a dividing cell can also be observed as the similar picture as the cytoplasmic membrane. Photo 2 is the picture of *Sal. typhi 57* showing the same staining as in *B. subtilis*. Photo 3 is of *Staph. aureus*, gram-positive

Photo 1. *B. subtilis*, Culture and staining, see text.
Photo 2. *Sal. typhi* 57, Culture and staining, see text.
Photo 3. *Staph. aureus*, Culture and staining, see text.
Photo 4. Plasmolyzed *B. subtilis*, Culture and staining see text.


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coccus, and shows the similar staining properties as the gram negative bacterial cells studied; i. e., one to two nuclear apparatuses can be seen in a cell and the transverse septum in the dividing cell is recognized between two nuclei. Photo 4 shows *B. subtilis* cells plasmolyzed by exposing to ether vapor and stained with Victoria blue 4R after treating with mercuric chloride. The cytoplasmic membrane is stained bluish purple and the cell wall is faintly visible outside of the membrane, showing that Victoria blue 4R stains cytoplasmic membrane but not the cell wall.

SUMMARY

A selective and simultaneous staining method for nuclear apparatus and cytoplasmic membrane of some bacteria has been presented. Nuclear apparatus is stained with basic fuchsin after hydrolysis with 1 *N* HCl and cytoplasmic membrane is restained with Victoria blue 4R after treating with saturated mercuric chloride. By this method, the nuclear apparatuses of *B. subtilis*, *Sal. typhi 57* and *Staph. aureus* were stained red, and the cytoplasmic membrane and septum bluish purple distinctly. Thus this staining method would be of a great advantage in displaying the cellular structures of the bacteria

ACKNOWLEDGEMENT

The author wishes to express his appreciation to Prof. Murakami for his guidance and to Dr. Okuma and Miss Kuroda for their technical assistances.

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