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Mesosomes are a definite event in antibiotic-treated *Staphylococcus aureus* ATCC 25923

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Abstract: Mesosomes of *Staphylococcus aureus* ATCC 25923 treated with antibiotics were examined morphologically under the electron microscope. The Transmission Electron Microscope Rapid Method was used to eliminate the artifacts due to sample processing. Mesosomes were seen in all the antibiotic treated bacteria and not in the control group. The main factor that contributes to the formation of mesosomes in the bacteria was the mode of action of the antibiotics. The continuous cytoplasmic membrane with infolding (mesosomes) as in the *S. aureus* ATCC 25923 is therefore confirmed as a definite pattern of membrane organization in gram positive bacteria assaulted by amikacin, gentamicin, ciprofloxacin, vancomycin and oxacillin antibiotics. Our preliminary results show oxacillin and vancomycin treated bacteria seemed to have deeper and more mesosomes than those treated with amikacin, gentamicin and ciprofloxacin. Further research is needed to ascertain whether the deep invagination and the number of mesosomes formed is associated with the types of antibiotic used.

INTRODUCTION

The first electron micrograph of the ultra thin section of a bacterial cell has been devoted to the elucidation of the fine structure of the bacterial cell. However, the technical difficulties and variant of species of bacteria have limited the scientific world from studying in detail the fine structures of the bacteria, such as the outer membrane, plasma membrane and mesosomes (Hackenberg, 2005). In this study, the focus will be on the mesosomes of the bacteria. Based on their morphological and ultra structure aspects, the mesosomes can be classified as membrane-bounded organelle. This structure could be an extended region of the plasma membrane. There is now general agreement that mesosomes are present in both gram-positive and gram-negative bacteria (Sidaway, 1980).

The focus of this study is to prove that the mesosomes are a real event. The bacteria will be exposed to five different kinds of antibiotics. The antibiotics selected are based on their mode of action. The Transmission Electron Microscope Rapid Method (TEMRM) would be applied in the processing of the control and antibiotic-treated bacteria groups. The main idea of using TEMRM is to avoid the presence of mesosomes due to the preparation technique of electron microscopy. The TEMRM was utilized based on the assumption that bacteria being a prokaryocyte would be fixed, but the fixation would not stop the living process of the bacteria.

MATERIALS AND METHODS

Bacteria culture

S. aureus ATCC 25923 was obtained from the Bacteriology Unit, Institute for Medical Research, Kuala Lumpur. The concentration of the bacteria used in this study was 1.4 X 10^7 bacteria per test and the ratio between the bacteria and the fixative solution was at 1:15.

Disc diffusion test

A suspension of *S. aureus* ATCC 25923 at 1.0 x 10^8 CFU/ml was made and streaked in at least three directions over the surface of the Mueller-Hinton agar to obtain a uniform growth. After the plates were dried for five minutes, antibiotic disks of ciprofloxacin, amikacin, oxacillin, gentamicin, and vancomycin were placed on the swabbed agar. The plates were incubated overnight at 37°C. Following incubation, the bacteria around the rim of inhibition growth zone around each disk were used for electron microscope analysis.

Transmission electron microscope rapid method

The bacteria that were fixed for 20 minutes in the 2% glutaraldehyde (GA) in 0.1M PBS were washed with distilled water (X6). After staining with 2% uranyl acetate (UA) for 5 minutes, the bacteria were exposed to osmium tetraoxide (OT) for 5 minutes. The dehydration of the exposed bacteria to OT was dehydrated with a series of acetone (50%, 70%, 90% and absolute acetone) respectively for 5 minutes each. Polymerization was done with pure epoxy resin in an embedding oven at 90°C for 2 hours after the bacteria have been infiltrated by a mixture of acetone and epoxy resin (1: 1) for 15 minutes. The blocks were trimmed and cut to 90 nm ultra thin sections and mounted on 200 mesh thin bar copper grids. The specimens were then stained with Reynold's stain for 1 minute. Each specimen was examined at 65 000X and 110 000 X magnifications by using Tecnai G2 TEM at an accelerating voltage of 90 KV.

RESULTS AND DISCUSSION

A total of 60 electron micrographs were taken for analyzing the presence of mesosomes in the bacteria. The mesosomes were only present in the antibiotics treated bacteria (Figures 1B, 1C, 1D, 1E and 1F) and not in the control group (Figure 1A). The vancomycin and oxacillin treated bacteria (Figures 1E and 1F) shows a deep invagination of mesosomes compared to amikacin, gentamicin and ciprofloxacin treated bacteria (Figures 1B, 1C and 1D). The number of mesosomes was more in the vancomycin and oxacillin treated bacteria (e.g. two or more mesosomes were seen in Figures 1E and 1F) than amikacin, gentamicin and ciprofloxacin treated bacteria (only one mesosome seen in Figures 1B, 1C and 1D). Moreover, amikacin, gentamicin and ciprofloxacin treated bacteria had much thicker outer membrane than vancomycin and oxacillin treated bacteria (Figures 1B, 1C and 1D compared to Figures 1E and 1F).

This study proves that the formation of mesosomes were indeed a real event, based on the observations of the presence of mesosomes only in the antibiotic-treated *S. aureus* ATCC 25923 (Figures 1B, 1C, 1D, 1E and 1F). The formation of mesosomes in bacteria was to act as a defensive mechanism to protect the bacteria from antibiotic assault, thereby sustaining the survival of the species. It is also interesting to note that the plasma membrane of the bacteria act as mitochondria of eukaryocytes (Pichler *et al.*, 2001). Thorne and Barker (1969) successfully isolated certain enzymes, such as ATPase, acetokinase and phosphotransacetylase from plasma membrane and mesosomes of *Lactobacillus casei*. These same enzymes can also be found in the mitochondria of the eukaryocytes (Pichler *et al.*, 2001). The association between the mesosomes and mitochondria was also supported by the detection of lipoteichoic acid (the end product of the wall synthesizing enzymes) in all of these

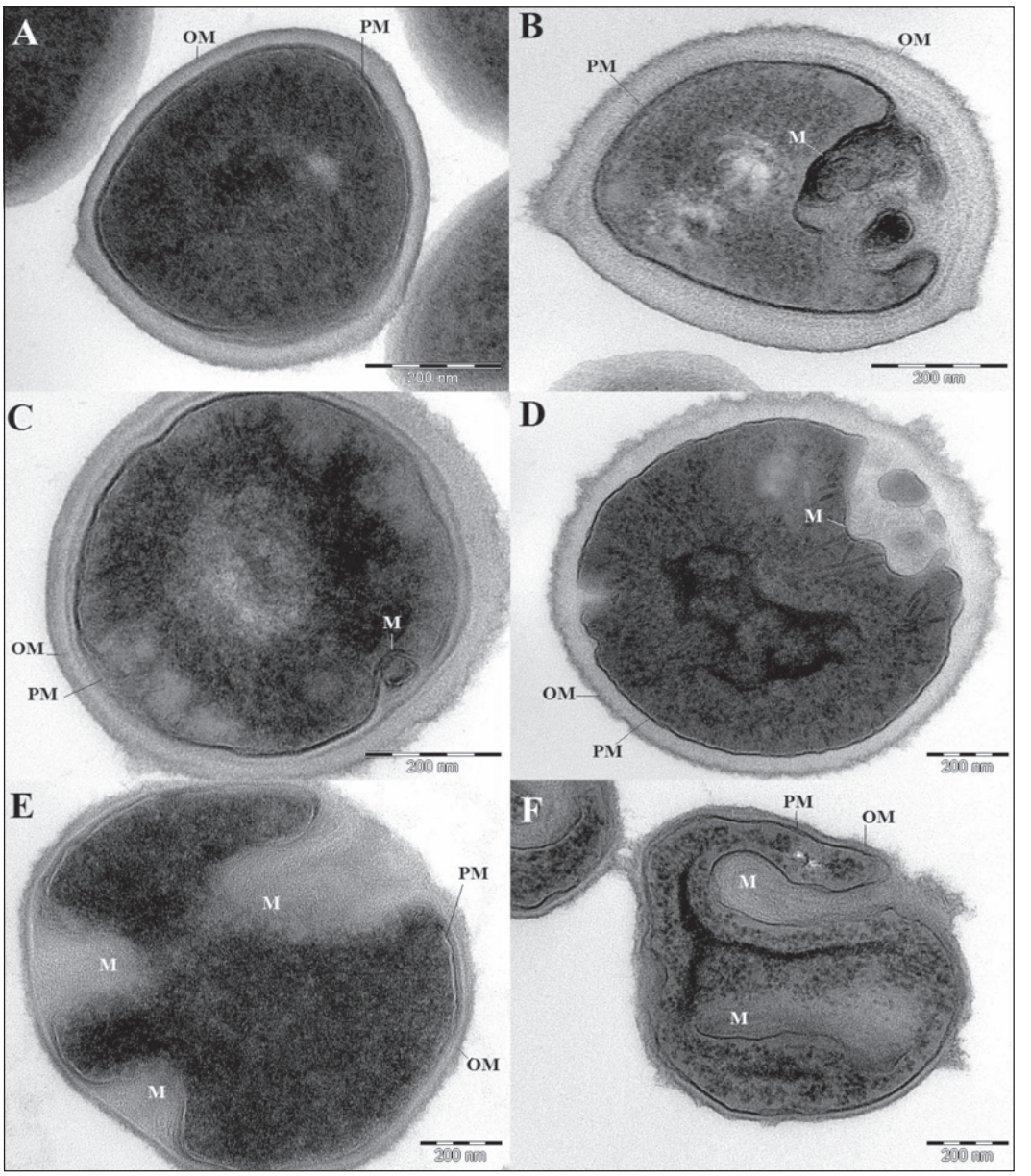

Figure 1. The electron micrographs : (A) = control group, (B) = amikacin treated bacteria, (C) = gentamicin treated bacteria, (D) = ciprofloxacin treated bacteria, (E) = oxacillin treated bacteria and (F) = vancomycin treated bacteria. (E) and (F) electron micrographs show deep invagination of mesosomes in the bacteria compared to (B), (C) and (D) electron micrographs. (A) electron micrograph of the control group did not show any mesosomes in the bacteria. M = Mesosomes, PM = Plasma Membrane and OM = Outer membrane. Magnification of (A), (B) and (C) are at 111 000X and magnification of (D), (E) and (F) are at 65 000X. Scale bar is at 200 nm.

organelles (Greenawalt & Whiteside, 1975).

It was also reported the mitochondria of the rat liver could be disassembled to smaller constituent structures, such as the lamellae and tubules. This is related to the formation of mesosomes in the bacteria. The mitochondria of different species and cell types could react differently depending on the situation and functions of the

species and cell types. The harsh condition (the antibiotics environment as in this study) would concentrate the oxidative phosphorylation enzymes and make the plasma membrane of the bacteria more fluid to form the mesosomes. These mesosomes would increase the surface area to facilitate more efficiently the oxidative reaction (Greenawalt & Whiteside, 1975).

The study also revealed that membrane attacking antibiotic (such as the vancomycin and oxacillin) (Figures 1E and 1F), caused deep invagination of mesosome in the bacteria compared to the RNA and DNA attacking antibiotics (such as amikacin, gentamicin and ciprofloxacin) (Figures 1B, 1C and 1D). The number mesosomes present in vancomycin and oxacillin treated bacteria (Figures 1E and 1F) were more compared to the amikacin, gentamicin and ciprofloxacin treated bacteria (Figure 1B, Figure 1C and Figure 1D). These could be due to the frequencies of shutting down the existing channels in the membrane of the bacteria becoming more amplified in the vancomycin and oxacillin than the amikacin, gentamicin and ciprofloxacin treated bacteria. These amplifications would make the bacteria to be more active in the plasma membrane enzymes level and allowed the plasma membrane to be more fluid. The fluidity of the plasma membrane would allow the mesosomes to perturb deeper in the cytoplasm and increased the number of mesosomes in the bacteria. We also noticed that the amikacin, gentamicin and ciprofloxacin treated bacteria (Figures 1B, 1C and 1D) had thicker outer membrane than the vancomycin and oxacillin treated bacteria (Figures 1E and 1F). The thicker outer membrane's mesosomes were more confined to the plasma membrane than the thinner membrane's mesosomes. The confinement of the mesosomes to the plasma membrane allowed us to conclude that the thickeing of the outer membrane played a role in the fluidity of the plasma membrane and the formation of the mesosomes.

The same event that took place in antibiotics treated bacteria could happen in the bacteria in the fixative solutions because of the initial fixation by glutaraldehyde on the transepithelial Na^+ and K^+ currents. The research teams of Higgins and Daneo-Moore (1974) and Silva *et al*. (1976) had successfully demonstrated the formation of mesosomes caused by GA and OT fixations. We believe the duration of fixation they used may have an impact on the formation of the mesosomes. Thus, we modified the fixation time in TEMRM to the generation time of the *S. aureus* and thereby provided us the true descriptive analysis in the formation of mesosomes in the antibiotics treated conditions.

This study clearly shows that the mesosome observed in the antibiotics-treated *S. aureus* ATCC 25923 was a real event and the mesosomes could only be present if the surroundings were hostile to the bacteria's living condition. In order to avoid any misleading conclusion during any study of ultra structure analysis of bacteria, we should choose the right protocol of electron microscopy. Consideration must be given to generation time and how the bacteria species react to the fixative solutions. The TEMRM Method that was applied in this study confirmed that the formations of mesosomes in the bacteria were not due to the TEM preparation techniques but indeed a definite event. Further research is needed to ascertain whether the deep invagination and the number of mesosomes formed is associated with the types of antibiotic.

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