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Suppression of Protein A Production Decreases the Resistance of *Staphylococcus aureus* to the Bactericidal Effect of H_2O_2

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ABSTRACT. Using several strains of *Staphylococcus aureus,* the resistance to the bactericidal effect of H_2O_2 was investigated. The protein A production in the bacteria of Cowan I, Smith and 209P strains which are capable to produce protein A on BHIA could be effectively suppressed by the cultivation on MSA. The viability of the bacteria which suppressed the protein A production was decreased by the incubation with H_2O_2. The protein A deficient Wood 46 strain did not show any difference in survival rate between those grown on BHIA and MSA. These results suggested that protein A was related to the resistance to the bactericidal effect of H_2O_2.

Key words : protein A — *Staphylococcus aureus* — hydrogen peroxide — bactericidal effect

With respect to the function of the unique substance, protein A which is contained in the cell walls of *Staphylococcus aureus* (*S. aureus*), many workers suggested that protein A possessed the anti-phagocytic effect of polymorphonuclear leukocytes (PMN)[1-3] and that the bactericidal effect of PMN was displayed with hydrogen peroxide (H_2O_2).[4,5] Our previous study[6] indicated that the resistance of Cowan I strain to the killing effect of H_2O_2 was higher than that of Wood 46 strain, suggesting that the different response against the killing effect was due to the protein A content in the cell walls of both strains. This study was, however, carried out with the genetically different strains, Cowan I and wood 46, and the question arises as to whether protein A is really responsible for the resistant characteristic of *S. aureus* against the killing effects or not. In this study, several strains were grown under two different culture conditions, one allowed to produce normally protein A and the other to suppress the protein A production. The results are reported in this paper.

MATERIALS AND METHODS

Bacterial strains and culture methods

Four strains of *S. aureus* were used in this experiment. Cowan I and Wood 46 strains were kindly supplied by Dr. Y. Arai (Saitama Medical School, Saitama, Japan). The other two strains, Smith (IID803) and 209P (IID671) strains were

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purchased from the Type Culture Collection Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan. Each strain was cultured in two different media, one was the brain heart infusion agar (BHIA : Nissui Pharmaceutical Co., Tokyo) for the normal production of protein A and the other was the mannitol salt agar (MSA : Eiken Chemical Co., Tokyo) for the suppression of protein A production.[7] The bacteria cultured for 48 hr at 37°C were collected, washed twice and then suspended in PBS at approximately 10^9 cells/ml for the determination of the amount of protein A. For the assay of the resistance to H_2O_2, suspension at approximately 10^8 cells/ml was prepared.

Assay of protein A

The amount of protein A in the cell walls of each strain was reciprocally determined by a modified method of Winblad and Ericson,[8] and Spika et al.[9] Briefly, the bacteria in one-milliliter portion was spun down at 2,300× g for 15 min into a pellet. The bacteria were then suspended in 2.0 ml of PBS containing 2 μg of lysostaphin (Schwartz/Mann, Orangeburg, New York) per ml and incubated for 2 hr at 37°C. After incubation the suspension was centrifuged to remove nonlysed bacteria and cell debris. The supernatant was made up into serial two-fold dilutions in PBS on an agglutination tray (Tomy Seiko Co., Tokyo). To 200 μl of each diluted supernatant was added 200 μl of 2 per cent suspension of sensitized sheep erythrocytes (Denka Seiken Co., Tokyo). The mixture was incubated for 2 hr at 37°C and kept at 4°C overnight. The reciprocal hemagglutination titer was defined as the inverse of the last dilution which showed the visible hemagglutination.

Assay of the resistance to H_2O_2

The resistance of *S. aureus* to the killing effect of H_2O_2 was assayed as follows. The mixtures containing 2.0 ml bacteria suspension obtained from the culture and 2.0 ml H_2O_2 in PBS solution (30 μmol/ml or 60 μmol/ml) were incubated at 37°C in a water bath. After incubation (1 and 2 hr), a 0.5 ml portion of the mixture was serially 10-fold diluted with the saline and the viable cell number in the mixture was counted by the usual pour plate method. The results were expressed as per cent of the viable cell count, defined as the bacteria that could produce colonies on nutrient agar.

RESULTS

The changes of the protein A content in the bacteria under the different culture conditions are summarized in Table 1. The strains, such as Cowan I, Smith and 209P, which normally produce protein A on BHIA showed remarkable reduction in the protein A production on MSA which is well known as the suppressive culture medium of the protein A production, while no detectable change was observed in the protein A deficient Wood 46 strain under both culture conditions. These results indicated that the protein A production is efficiently controlled by the use of the culture media. The cultivation on MSA and BHIA was, therefore, used in the further experiments.

When the bacteria of Cowan I strain were cultivated on BHIA and MSA, and then exposed to H_2O_2, distinct differences in the viable cell count were observed. Fig. 1 demonstrates that the viability of the bacteria cultured on

TABLE 1. Hemagglutination titer of *S. aureus* strains cultured on BHIA and MSA

Strains	BHIA	MSA
Cowan I	128	64
Smith	32	8
209P	64	32
Wood 46	N.D.*	N.D.

Figures represent the reciprocal average values obtained from four separate experiments.
* Not detectable.

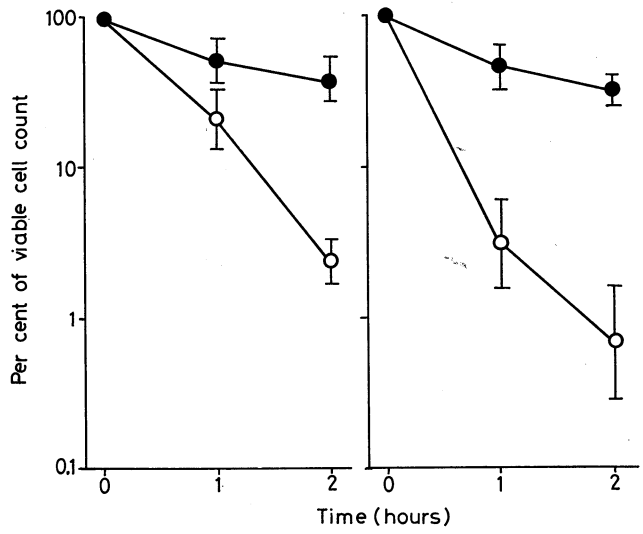

Fig. 1. The comparison of resistance between the organisms grown on brain heart infusion agar (BHIA : ●) and mannitol salt agar (MSA : ○). The incubation mixtures contained the bacterial cells together with different final concentration of H_2O_2, 15 μmol/ml (left) and 30 μmol/ml (right). The points represent mean values of three determinations $\pm$ 1SD.

MSA was remarkably decreased by the exposure to H_2O_2 at the different concentrations, and that the decrease at 30 μmol/ml of H_2O_2 was more severe than that at 15 μmol/ml. Similar results were obtained, when the organisms of Smith and 209P strains were cultivated on BHIA and MSA, and then exposed to H_2O_2 at 30 μmol/ml concentration (Fig. 2). When the organisms of Wood 46 strain grown on BHIA and MSA were similarly exposed to H_2O_2, the viability of both cultures was decreased up to less than 10 per cent and there was no distinct difference between the viable cell count of both culture groups (Fig. 3). These results indicate that the suppression of protein A production is related to the decrease of protective activity against the killing action of H_2O_2.

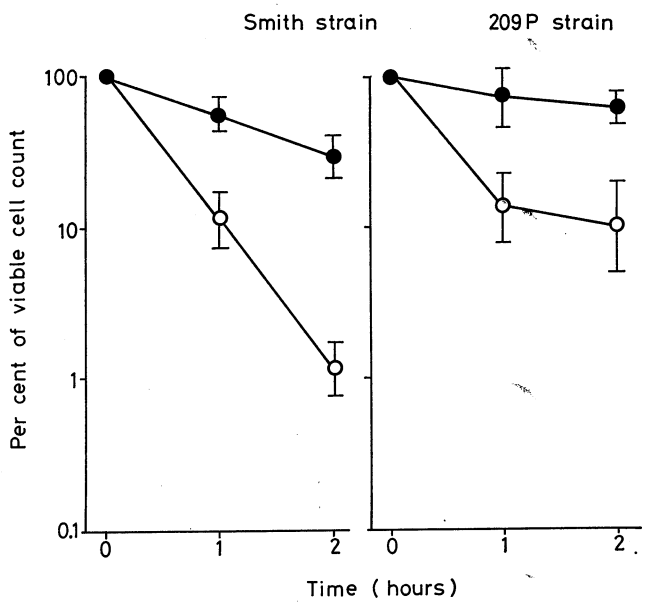

Fig. 2. The resistance of *S. aureus* strains to the killing effect of H_2O_2 Smith strain (left) 209P strain (right). The resistance to the killing effect of 30 μmol/ml H_2O_2 was compared between BHIA-cultures (●) and MSA-cultures (○) in each strain. The points represent mean values of three determinations ± 1SD.

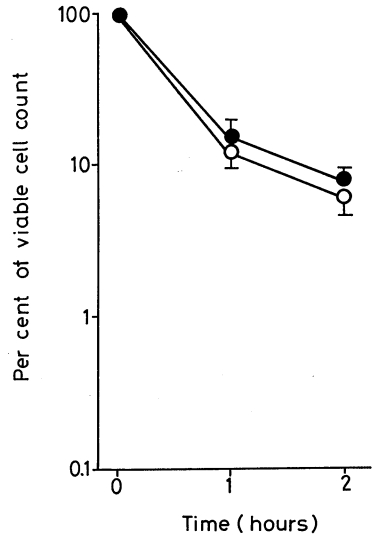

Fig. 3. The resistance of *S. aureus* Wood 46 strain to the killing effect of H_2O_2. For experimental details see Fig. 2.

DISCUSSION

The present author reported previously the different resistance between Cowan I and Wood 46 strains to the killing effect of H_2O_2.[6] However, as far as these strains that are different genetically are used, it may be hard to conclude that the resistance to the killing effect is due to the amount of protein A, because both strains may be different not only in the protein A production, but also in the other phenotypic characteristics responsible for the viability in H_2O_2. Nickerson et al.[7] reported that the protein A production of *S. aureus* was suppressed by the cultivation on MSA. Therefore, in the present experiment, the organisms which controlled the protein A production under the culture condition were used. The organisms of Cowan I, Smith and 209P strains cultured on BHIA were more resistant to the killing effect than those on MSA, but the resistance was not detected in the protein A deficient Wood 46 strain under the same conditions. The protein A content was determined by the hemagglutination titration method. The values obtained on the strains cultured on BHIA were, however, quite different each other, i.e., the hemagglutination titer of Cowan I strain was four times that of Smith and twice that of 209P strain. The viability of these three strains did not reflect the difference of their protein A content. However, the viability of the organisms under the suppressed condition (MSA cultures) was remarkably lower than that under the normal condition (BHIA cultures). Besides, these strains showed higher viability than that of the protein A deficient Wood 46 strain. Therefore, it seems that the resistance to the killing effect is related to the protein A content in the cell walls.

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