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Original Article

Electron Microscopic Studies on Bactericidal Effects of Electrolyzed Acidic Water on Bacteria Derived from Kendo Protective Equipment

Tetsuaki OSAFUNE[1], Tomoko EHARA[1,2] and Takashi ITO[3]

[1]*Department of Life Sciences, Nippon Sport Science University, Yokohama, Japan*
[2]*Department of Microbiology, Tokyo Medical University, Tokyo, Japan*
[3]*Department of Preventive Medicine and Public Health, Nippon Sport Science University, Tokyo, Japan*

Abstract

Objectives: Kendo protective equipment is used without washing for a long time. *Staphylococcus saprophyticus, Micrococcus luteus,* and *Bacillus sphaericus* are frequently isolated from the mask ('men' in Japanese) of kendo protective equipment during one year. To investigate the bactericidal effects of electrolyzed acidic water on these three bacteria, we observed their cellular structures by electron microscopy after treatment with such water.

Methods: Each bacterium isolated from 'men' was treated with electrolyzed acidic water and then observed under scanning and transmission electron microscopes.

Results: When *S. saprophyticus* was treated with electrolyzed acidic water and its cellular structures were observed under a transmission electron microscope, ghost cytoplasm was observed, in which no ribosomal granules or fibrous DNA structures were present, and the cell wall inner layer was detached from the outer layer. Under a scanning electron microscope, the structure of the cell wall surface layer was wrinkled, and round pores were partially formed, indicating that the cytoplasmic structures were flushed out of the cells treated with electrolyzed acidic water through the pores formed in the cell wall. In *M. luteus*, the destruction of ribosomal granules and that of DNA fibers were observed to be similar to those of *S. saprophyticus*. For *B. sphaericus*, the effect of electrolyzed acidic water was investigated using vegetative cells. A dissociation between the cytoplasm and cell wall wrinkled the cell surface layer.

Conclusion: On the basis of above findings, electrolyzed acidic water was found to destroy the cellular structures of the three bacterial species frequently isolated from kendo men within a short time. Electrolyzed acidic water may be useful for disinfecting of kendo equipment.

Key words: bacteria, electrolyzed acidic water, electron microscopy, kendo mask ('men' in Japanese)

Introduction

Infectious diseases caused by weakly toxic, non toxic, and non harmful bacteria, which have been considered to normally cause no infectious diseases in healthy individuals, have recently been reported (1). Human infections by these non pathogenic bacteria are called opportunistic infections. Many cases of opportunistic infections caused by non-pathogenic bacteria, such as *Pseudomonas aeruginosa*, which had been considered non pathogenic, and non-pathogenic epidermal *Staphylococcus* and *Enterococcus*, have occurred as a result of irradiation with electromagnetic waves, the administration of immunosupressors for organ transplantation, the use of anti-cancer drugs and adrenocortical hormones with advances in medical care, an increase in the number of AIDS patients whose immune system is weak, hard exercise, and decreased physical strength after disease (1–4). Thus, the analysis of environmental bacterial floras is important with regard to microbiology, public health, and the prevention of epidemics. At the same time, investigations of the inhibition mechanisms of bacterial proliferation and the bactericidal effects of various disinfectants and drugs for isolated and identified bacteria are urgently needed for the establishment of measures for bacterial eradication. To clarify the states of bacterial floras in natural environments, our laboratory has incorporated an advanced instrument, the auto-

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Reprint requests to: Tetsuaki OSAFUNE, D.Med.Sci., Ph.D.
Department of Life Sciences, Nippon Sport Science University, 1221 Kamosida, Yokohama 227-0033, Japan
TEL & FAX: +81(45)963-7935
E-mail: osafunet@nittai.ac.jp

matic bacterial test system, and performed detailed isolation and identification studies of bacteria found on educational and work facilities, exercise devices, exercise protection equipment, and uniforms. We have identified bacterial flora found on kendo equipment and judo and wrestling mats, as well as in pool water, sumo ring soil, and dance fields (5–11). Furthermore, we have been studying the *in vitro* bactericidal effects of various disinfectants on bacteria constituting the flora in various sport education facilities and sport equipment from hygienic, public health, and epidemiological viewpoints (5, 12).

Kendo is a contact sport in which players hit their opponents with bamboo swords and wear protective equipment including a kendo mask. Kendo protective equipment is used for a long time without washing, usually until the equipment becomes worn out (13). Thus, elucidating the bacterial flora present on kendo protective equipment including the men is important with regard to microbiology, hygiene, public health, and the prevention of epidemics. In addition, investigations of the bacterial proliferation inhibition mechanisms and antibacterial effects of various disinfectants against identified bacteria are urgently needed for sport education and the establishment of measures for bacterial eradication. In this study, to clarify the bactericidal effects of electrolyzed acidic water on three bacterial species present on men, namely *Staphylococcus saprophyticus*, *Micrococcus luteus*, and *Bacillus sphaericus*, isolated and identified at high frequencies throughout the year, changes in bacterial ultrastructure were observed by electron microscopy.

Materials and Methods

1. Sample collection

Twenty subjects were randomly selected from male and female members of a kendo club of University. The bacterial flora on their kendo masks was sampled and studied investigated for one year. After kendo classes and practice, kendo protective equipment is usually stored in a gymnasium locker at room temperature (20–25°C) without periodic disinfection or sun exposure. For sample collection, a Countact (Biomerieux Japan) was placed on the inner surface of the left jaw region of a 'men' immediately after kendo practice, and the entire Countact was pressed with 500 *g* of pressure for 10 sec using a Countact applicator (14).

2. Culture method

The sample collected on the Countact was cultured at 37°C for 24 h in an incubator followed by culture at room temperature for three days (8). The morphology and color of the colonies collected on the Countact were observed, and the colonies were subjected to isolation culture using heart infusion agar medium. Isolated bacteria were subjected to pure culture on heart infusion slants.

3. Identification using automatic bacterial test system

Each pure bacterial culture was Gram-stained. Then, the cell morphology of each culture was observed, and the Gram stain result was observed under a light microscope (Nikon E500). Cards for identification (bioMérieux, Inc., France) were selected on the basis of Gram stain reactions. After initial classification, the bacteria were suspended in physiological saline and the resulting solution was placed in the card or ATB plate (bioMérieux, Inc., France). The card for identification was computer-processed using VITEK AMS and ATB automatic bacterial test systems (bioMérieux-Vitek Japan, Ltd.) and the bacteria were automatically identified after 4–48 h (15).

4. Samples of bacterial strains

Among the bacteria on the 'men' identified by the rapid automatic bacterial test system, *S. saprophyticus*, *M. luteus*, and *B. sphaericus*, which were detected at high frequencies throughout the year, were selected. *Staphylococcus saprophyticus* and *B. sphaericus* were cultured with shaking in nutrient broth at 37°C for 24 h, and *M. luteus* was cultured in heart infusion broth (Nissui Pharm.) under the same conditions (7, 14).

5. Treatment of bacteria with electrolyzed acidic water

Each bacterium isolated from the 'men' was suspended in 0.5% NaCl solution and adjusted to 10^{6-7} cells/ml. The bacterial suspension (0.2 ml) was added to a test tube containing 1.8 ml of electrolyzed acidic water (Oxilyzer, Koken Co.) (pH 2.7 or lower, oxidation-reduction potential: +1,100 mV, dissolved chlorine: 20–60 ppm), left to react at 20°C for 0.5 or 10 min, and diluted (14, 16). The samples were cultured on nutrient agar medium at 37°C for 24 h, and the viable bacterial cell concentration after treatment with electrolyzed acidic water was determined from the colony count on the medium.

6. Transmission electron microscopy

Each bacterium taken from the culture was fixed with 2.5% glutaraldehyde (TAAB) for 1 h at 4°C and then postfixed with 1% osmium tetroxide (EM Science, USA) for 1 h at 4°C (17). The suspension was then centrifuged and the resulting pellet was embedded in 2% agarose (Nakarai Co.) followed by dehydration in a 50–100% graded ethanol series and then dehydration in 100% acetone (Wako Pure Chem. Co.). The samples were embedded in Spurr's resin (EM Science, USA) and subjected to serial sectioning using a Leica UCT microtome. Sections were stained with 3% uranyl acetate (Merck, Germany) at room temperature followed by staining with lead citrate, after which they were examined with a JEOL CX-100 electron microscope (16).

7. Scanning electron microscopy

Each bacterium was washed with 0.1 M phosphate buffer, and fixed with 1% glutaraldehyde (TAAB). The sample was then dehydrated in graded ethanol series, and subjected to critical-point drying using isoamyl acetate (Wako Pure Chem. Co.) as intermediate solution and liquefied carbonic acid gas as transfer solution (10, 12). After drying, the sample was subjected to vacuum double-vapor deposition of carbon and gold (JEOL JFC-1600), and observed at an accelerating voltage of 10 kV under a scanning electron microscope (JEOL, JMS6040LV).

Results

The bacteria from the 'men' were analyzed using the automatic bacterial test system, and about 40 major species were isolated and identified throughout the year. Gram-negative rods were most frequently isolated, followed by Gram-positive rods and Gram-positive cocci. No Gram-negative coccus was isolated from the 'men' within the range investigated. Thirteen *Bacillus* species were isolated from the 'men'. *Bacillus sphaericus* was isolated at a high frequency during the year, but *B. subtilis*, *B. circulans*, *B. pumilus*, *B. thuringiensis*, *B. megaterium*, *B. cereus*, *B. lentus*, *B. licheniformis*, *B. macerans*, *B. alvei*, *B. laterosporus* and *B. coagulans* were isolated at low frequencies from the 'men'. The bacteria identified included the pathogenic etiological agent of *Vibrio enteritis* and *Vibrio parahaemolyticus*.

Table 1 shows the three bacterial species isolated from the 'men' at a high frequency. *Bacillus saprophyticus* was most frequently isolated among the *Bacillus* species, and the frequency was 37%. The frequencies of *Micrococcus luteus* and *B. sphaericus* were 34% and 17%, respectively.

Figure 1 shows the changes in the frequencies of the three most frequently isolated species, namely *S. saprophyticus*, *M. luteus*, and *B. sphaericus*, during the year. The frequencies of *S. saprophyticus* and *B. sphaericus* increased during summer (Fig. 1). The detection rates for *S. saprophyticus* and *B. sphaericus* in September were 60% and 30%, respectively (Fig. 1). In

Table 1 *Staphylococcus saprophyticus*, *Micrococcus luteus* and *Bacillus sphaericus* species were isolated from 'men' at a high frequency

Bacterium	Number of 'Men'		Frequency (%)
	Tested	Positive for bacterium	
Staphylococcus saprophyticus	180	67	37
Micrococcus luteus	180	61	34
Bacillus sphaericus	180	31	17

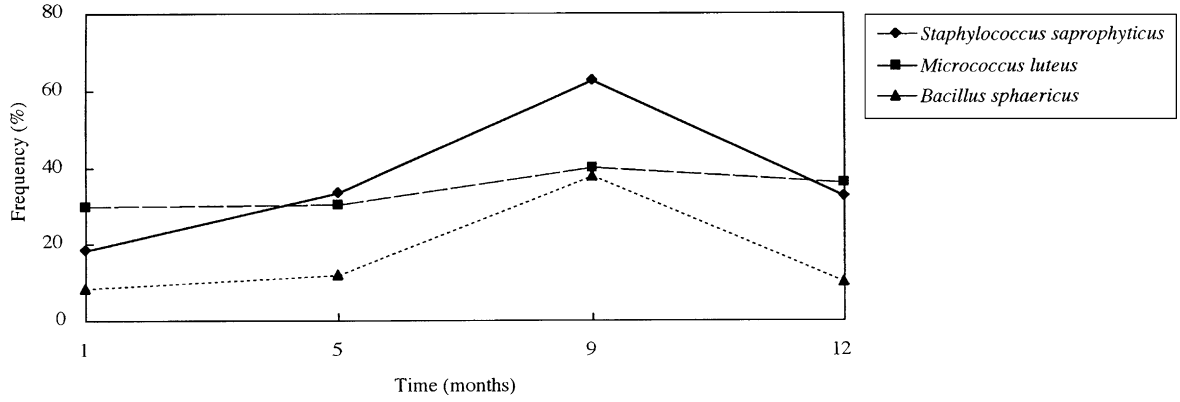

Fig. 1 Changes in frequencies of three most frequently isolated species, namely *Staphylococcus saprophyticus*, *Micrococcus luteus* and *Bacillus sphaericus* during year.

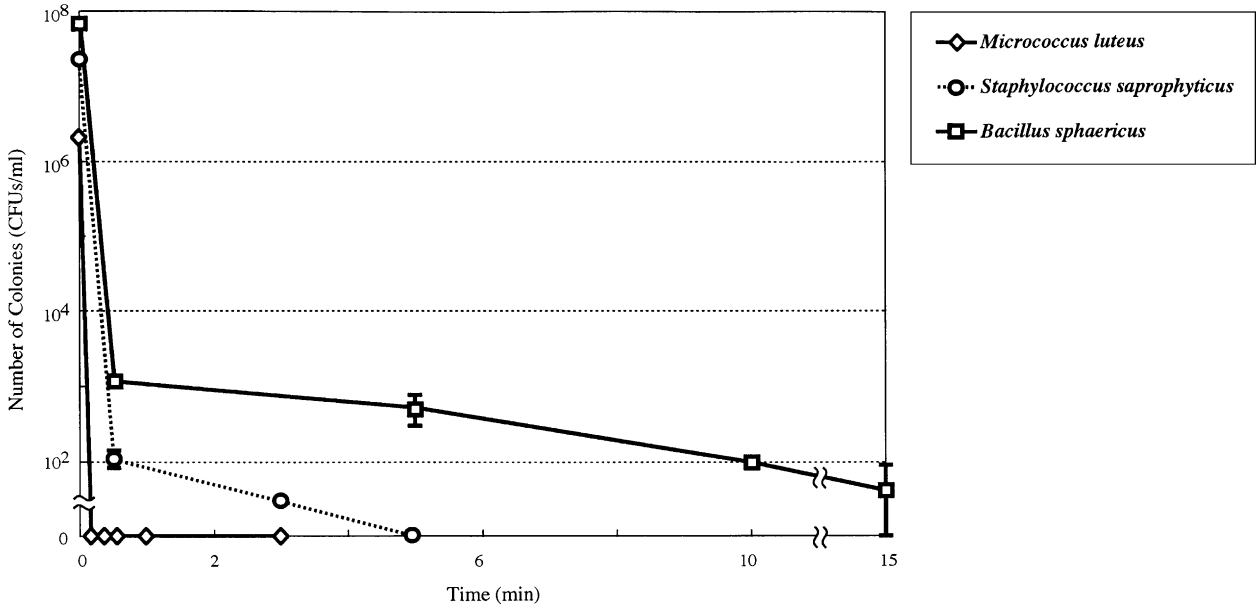

Fig. 2 Bactericidal effects of electrolyzed acidic water on *Staphylococcus saprophyticus*, *Micrococcus luteus* and *Bacillus sphaericus*. No growth was observed after treatment with hydrolyzed acidic water at 20°C for 0.5 minites for *M. luteus* and 5 min for *S. saprophyticus*. For *B. sphaericus*, the number of viable cells represented about 0.001% of the initial number of cells after 0.5 min and less than 0.0001% after 15 min.

contrast, the frequency of *M. luteus* was constant throughout the year, showing that this *M. luteus* is the most common bacteria in the bacterial flora on the 'men' (Fig. 1); thus, and *Micrococcus luteus* can be called the bacteria of the kendo mask.

The treatment of the three bacterial species with electrolyzed acidic water as disinfectant at 20°C for 10 min showed a strong bactericidal effect. Figure 2 shows the time course of the bactericidal effect of electrolyzed acidic water on *Staphylococcus saprophyticus*, *Micrococcus luteus* and *Bacillus sphaericus*. No growth was observed after treatment with hydrolyzed acidic water at 20°C for 0.5 min for *M. luteus* and 5 min for *S. saprophyticus*. For *B. sphaericus*, the number of survivors represented about 0.001% of the initial number of cells after 0.5 min and less than 0.0001% after 15 min (Fig. 2). This is in good agreement with the electron micrographs (Figs. 3, 4 and 5). Note that the growth of *Bacillus sphaericus* was not entirely

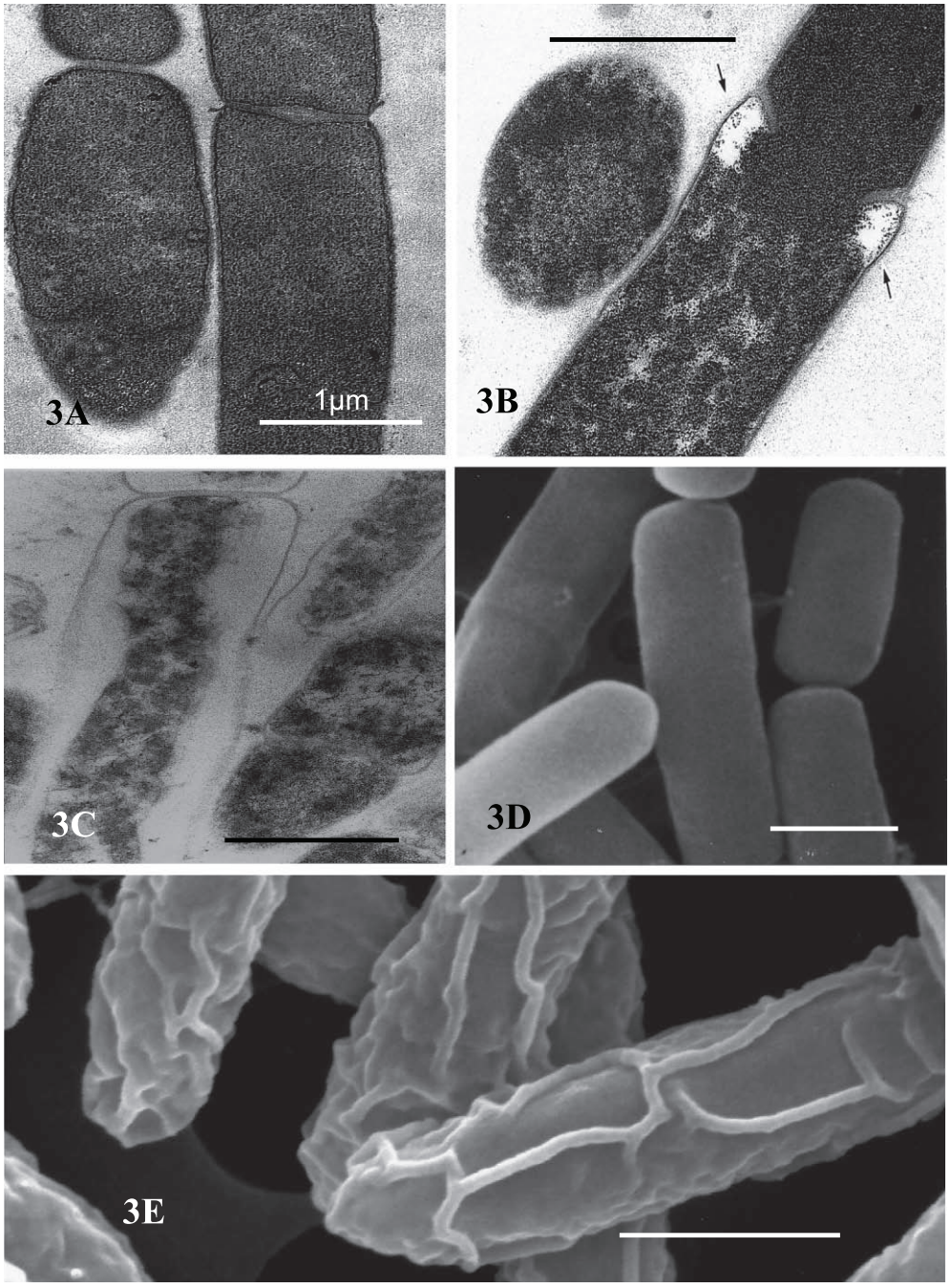

Fig. 3 A, B and C: Transmission electron micrographs of *Bacillus sphaericus*. Bar=1 μm. D and E: Scanning electron micrographs of *Bacillus sphaericus*. Bar=1 μm. A: *Bacillus sphaericus* cells were double-fixed with 2.5% glutaraldehyde and 1% osmium tetroxide fixatives. B: Treatment of *B. sphaericus* cells with hydrolyzed acidic water at 20°C for 0.5 minutes decreased cytoplasmic density. The arrows indicate a decrease in electron density in the septum region. C: Treatment of *B. sphaericus* with hydrolyzed acidic water at 20°C for 10 minutes decreased cytoplasmic density and separated the cytoplasm from the cell wall. Bar=1 μm. D: Scanning electron microscopic observation of *B. sphaericus* before treatment with hydrolyzed acidic water. E: Bacillus sphaericus after treatment with hydrolyzed acidic water observed under scanning electron microscope.

inhibited at 20°C for a 15-min treatment with hydrolyzed acidic water (Fig. 2).

Figure 3A shows ultrathin sections of *B. sphaericus* that was isolated from the 'men' and observed under an electron microscope. The cells were that double-fixed with 2.5% glutaraldehyde and 1% osmium tetroxide fixatives. *Bacillus sphaericus* cells were rod-shaped with a normal cell wall and a normal cell membrane, and ribosomal granules, DNA and a septum were noted in regions with decreased electron density. A cross section of the spherical morphology is shown in Fig. 3A. Figure 3B shows ultrathin sections of *B. sphaericus* treated with electrolyzed acidic water at 20°C for 0.5 min as observed under a transmission electron microscope. The arrows in Fig. 3B indicate a decrease in electron density in the septum region of the mitotic surface. Treatment of *B. sphaericus* with electrolyzed acidic water at 20°C for 10 min decreased cytoplasmic density, separated the cytoplasm from the cell wall, and formed spaces in the cells, which clearly show ghost cytoplasm (Fig. 3C). Cells were partially destroyed, and electron density decreased owing to the outflow of cytoplasm. Figure 3D shows a scanning electron microscopic observation of *B. sphaericus* before treatment with electrolyzed acidic water. The morphology of the vegetative cell was a rod, and the cell wall surface structure was smooth. Figure 3E shows *B. sphaericus* after treatment with electrolyzed acidic water as observed under a scanning electron microscope. The cell wall was cracked and markedly shrank (Fig. 3E).

The bactericidal effect of electrolyzed acidic water on *S. saprophyticus* was investigated. No cell proliferation was noted after treating the cells at 20°C for 10 min. Figure 4A shows an electron microscopic observation of an ultrathin section of *S. saprophyticus* cells without treatment with electrolyzed acidic water. *Saphylococcus saporophyticus* cells with a normal cell wall, a cell membrane, ribosomal granules, and DNA are observed in the section (Fig. 4A). Figure 4B shows the section as observed under a scanning electron microscope. The cells were treated with electrolyzed acidic water, and the effect of such treatment on the ultrastructures were observed under a

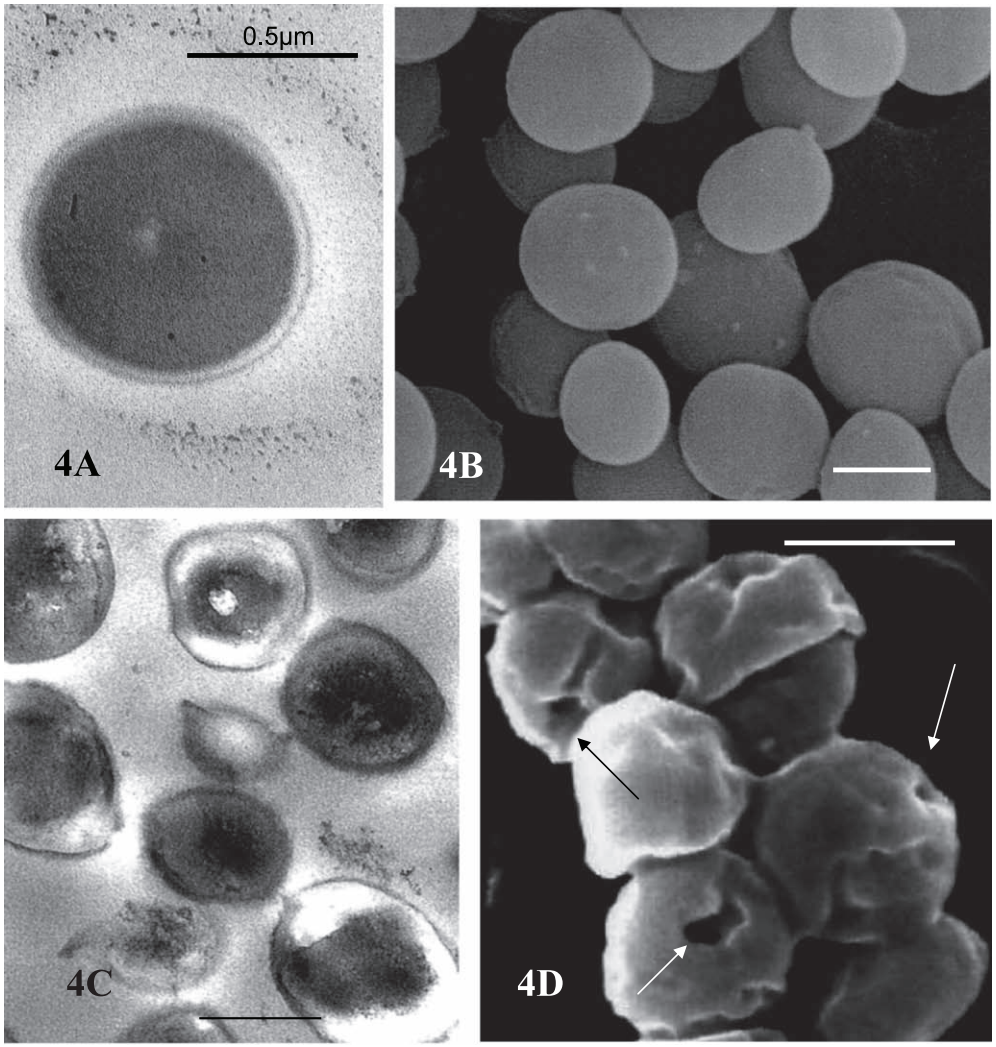

Fig. 4 A and C: Transmission electron micrographs of *Staphylococcus saprophyticus*. Bar=0.5 μm. B and D: Scanning electron micrographs of *Staphylococcus saprophyticus*. Bar=0.5 μm. A: *Staphylococcus saprophyticus* cells before treatment with hydrolyzed acidic water. C: *Staphylococcus saprophyticus* cells were treated with hydrolyzed acidic water, and the effect of such treatment on the ultrastructure was observed. B: *Staphylococcus saprophyticus* cells were before treatment with hydrolyzed acidic water. D: *Staphylococcus saprophyticus* treated with hydrolyzed acidic water. The arrows show that the structures of cell wall shrank and partially cracked.

transmission electron microscope (Fig. 4C). As observed in Fig. 4C, ribosomal granules and DNA were absent in the cells. However, the external morphology of *S. saprophyticus* showed a thick cell wall as in the control, showing that the characteristic spherical structure of *Staphylococcus* was maintained (Fig. 4C). In the ultrathin section shown in Fig. 4C, the surface layer of the cell wall was partially detached. Figure 4D shows *Staphylococcus saprophyticus* observed by scanning electron microscopy. The cell wall shrank and partially cracked shown by the arrows, resembling that of unicellular green algae. These findings were consistent with the transmission electron microscopy findings in the region with the detachment of the surface layer.

Figures 5A and 5B show ultrathin sections of a tetrad, *Micrococcus luteus*, observed under a transmission electron microscope. A normal cell wall, ribosomal granules, DNA, and a new septum were observed in the *M. luteus* cells (Fig. 5A). Figure 5C shows the *M. luteus* cells observed under a scanning electron microscope. The surface layer was smooth, and no deformation of the cell wall was observed (Fig. 5C). Figures 5B and 5D show *M. luteus* cells treated with electrolyzed acidic water for the observation of bactericidal effect. Treatment with electrolyzed acidic water rapidly decreased the viable *M. luteus* cell count (Fig. 2). As in *S. saprophyticus*, electrolyzed acidic water induced the appearance of ghost cells with decreased electron density owing to the deformation of the cell wall and the loss of cytoplasm in *M. luteus* cells, structurally showing a bactericidal effect (Fig. 5B). Figure 5D shows *M. luteus* cells treated with electrolyzed acidic water observed under a scanning electron microscope. Compared with the control shown in Fig. 5C, the cell wall structure was markedly deformed (Fig. 5D).

Discussion

Bacteria are classified into strongly toxic bacteria with pathogenicity and weakly toxic bacteria (18, 19). The frequency occurrence of opportunistic infections has increased with advances in medical care, for example, infection with weakly toxic bacteria often occurs in patients with organ transplants, infectious diseases complicating AIDS, and decreased resistance to infections as a result of the administrations of adernocortical hormones, anticancer drugs, and immunosupressors

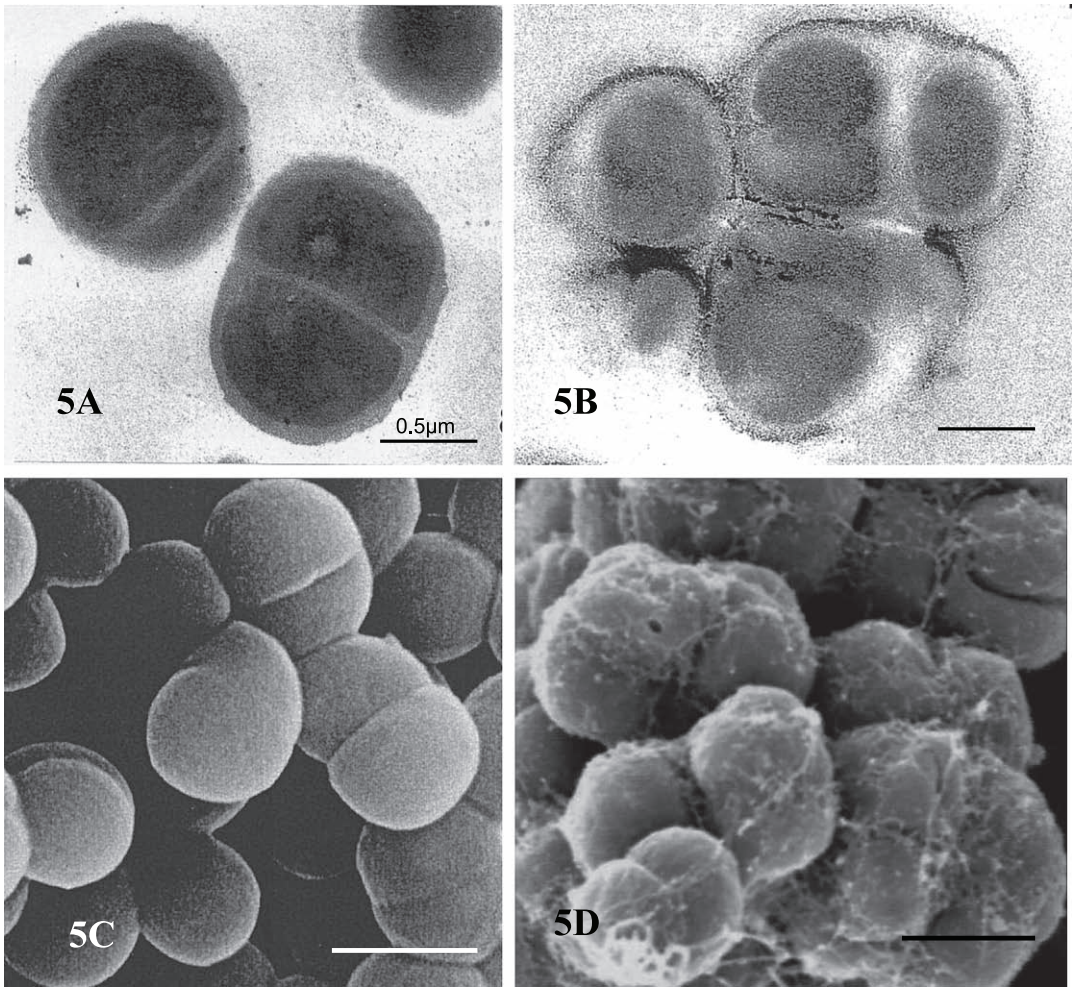

Fig. 5 A and B: Transmission electron micrographs of *Micrococcus luteus*. Bar=0.5 μm. C and D: Scanning electron micrographs of *Micrococcus luteus*. Bar=0.5 μm. A: Transmission electron micrographs of *M. luteus*. B: Treatment of *M. luteus* cells with hydrolyzed acidic water decreased cytoplasmic density. C: Scanning electron micrographs of *M. luteus* before treatment with hydrolyzed acidic water. D: *Micrococcus luteus* cells after treatment with hydrolyzed acidic water observed under scanning electron microscope. The structures of the cell wall markedly deformed and shrank.

for organ transplantation (20). Infectious diseases caused by nonpathogenic bacteria and weakly toxic bacteria that do not occur in healthy individuals, and opportunistic infections by many weakly toxic bacteria, such as *Staphylococcus*, *Pseudomonas*, and *Corynebacterium* species, and fungi have become social problems (19, 20). Under such circumstances, we consider that a prior understanding of the state of bacterial floras in environments is important for education and the prevention of epidemics, and for general basic knowledge of teachers and students.

Kendo players wear 'men', 'do' (body armor), and 'kote' (hand guard) to protect the body. This equipment is used without washing for a long time, usually until it is worn-out. In 1994, Tabuchi measured the number of bacterial cells adhered to the jaw region of 'men' by colony counting, and investigated measures for the eradication of bacteria from protective equipment (13). Tanaka et al. (14) isolated and identified bacteria from 'men' throughout a year using a rapid automatic bacterial test system, and initially identified the bacterial flora on the 'men'. Furthermore, the bactericidal effects of various disinfectants on the bacteria constituting the flora have been studied with regard to hygiene, public health, and epidemiology (7).

In this study, we examined *S. saprophyticus*, *M. luteus*, and *B. sphaericus* from the bacteria specific to the bacterial flora on 'men' because these bacteria were frequently isolated. We treated these bacteria with electrolyzed acidic water and observed changes in their microstructures by electron microscopy to clarify the effects of electrolyzed acidic water. About 40 bacterial species were isolated on the 'men' and they belonged to the genera *Bacillus*, *Flavimonas*, *Chryseomonas*, *Acinobacillus*, *Stenotrophomonas*, *Corynebacterium*, *Comamonas*, *Flavobacterium*, *Micrococcus*, *Pasteurella*, *Pantea*, *Pseudomonas*, *Vibrio*, *Eikenella*, *Sphingobacterium*, *Staphylococcus*, and *Acinetobacter*. Among these, Gram-positive cocci, *Staphylococcus* and *Micrococcus*, and Gram-positive rods, namely, *Bacillus*, were frequently isolated throughout the year (14). *Staphylococcus saprophyticus* was the most frequently identified organisum and, accounted for 37% of all the isolates, followed by *M. luteus*, which accounted for 34% (Table 1). *Micrococcus luteus* was detected at an almost constant rate throughout the year, showing that *M. luteus* is the most common bacteria detected on the 'men'. *Bacillus sphaericus* accounted for 17%. As shown in Fig. 1, *B. sphaericus* was frequently identified throughout the year and is considered to be "one of the bacterial species in 'men' ". Twelve other bacterial species of *Bacillus* were isolated. Many *Bacillus* species are nonpathogenic and widely distributed in nature, in which they form resistant spores. The bacteria change from the vegetative cells to the durable spore in culture at 37°C for 24 h after being maintained at room temperature (20–25°C) for 7–10 days (23). As shown in Fig. 2, the growth of *B. sphaericus* was not entirely inhibited at 20°C for 15-min treatment with hydrolyzed acidic water. *Bacillus sphaericus* cells can endure severe environments and are highly resistant to disinfection, dryness, and heat (23). The high numbers of *Bacillus* may reflect the characteristics of bacterial species in this genus. *Bacillus cereus* was isolated and identified. *B. cereus* has been reported to cause opportunistic infections such as sepsis, bronchial pneumonia, meningitis, and panophthalmitis. Patients with food poisoning caused by *B. cereus* showing diarrhea, abdominal pain, and vomiting have been reported in western countries and Japan (24, 25). Thus, *B. cereus* has attracted attention as a species that cause food poisoning (25).

Figure 1 shows changes in isolation frequency throughout one year. The frequencies of *S. saprophyticus* and *B. sphaericus* increased during summer. *B. sphaericus* produces a foul odor, and this may be the cause of the bad smell of 'men' during summer. The reasons for the high frequencies of isolation and colonization (flora) of these bacteria on the 'men' are important and need to be investigated.

Electrolyzed acidic water is safe for human use, but has a rapidly acting relatively wide antibacterial effect. The electron micrographs shown in Figs. 3B, 3C and 3E were taken to ultra structurally investigate the bactericidal effect of electrolyzed acidic water. A vegetative cell of *B. sphaericus* was thin-sectioned and observed by electron microscopy. The rod-shape and cell structure were maintained, and the cell membrane was also clearly observed, but a decrease in cytoplasmic electron density was found in the septum region as seen in Fig. 3B. In addition, linear structures with a low electron density were observed toward the inner side of the cytoplasm. In the cells shown in Fig. 3C, the cytoplasm was markedly altered, and the cytoplasm and cell wall were separated. The cell membrane and cell wall were partially wavy, showing good consistency with the findings of transmission and scanning electron microscopes (Figs. 3C and 3E). The structures were markedly damaged in *B. sphaericus* treated with electrolyzed acidic water.

The bactericidal effect of electrolyzed acidic water on *Staphylococcus saprophyticus* was investigated (Figs. 4A–D). Tanaka et al. (12) reported the bactericidal effect of treatment with 20–100% ethanol at 37°C for 15 min. The viable *S. saprophyticus* cell count rapidly decreased at concentrations up to 40%, and the proliferation rate of the bacteria treated with 40% ethanol was 0% (14). Based on the proliferation test in which the removal in ethanol of *S. saprophyticus* under these conditions was carried out, the finding may have been due to the bactericidal effect of 40% ethanol; therefore, the effects of ethanol treatment on morphology were observed by electron microscopy (12). *Staphylococcus saprophyticus* was cultured at 37°C for 24 h and observed under an electron microscope. In cells before the initiation of division, ghost inner structures were observed; cytoplasmic ribosomal granules were markedly lost, and no fibrous structure of DNA was observed. Moreover, ribosomal granules and DNA fibers were destroyed, and the outer layer of the cell wall was detached. These cellular structures are initiated by the stepwise separation of the cell wall from the outer layer with the exposure of the cells to ethanol with time (12). The spherical morphology of *S. saprophyticus* was maintained even though ghost cytoplasm occurred in the cells. The electron microscopy findings of treatment with hydrolyzed acidic water in our study (Figs. 4C and 4D) were similar to those of ethanol treatment in Tanaka et al. (12). In the scanning electron micrograph shown in Fig. 4D, pores were observed in one part of the cell wall, indicating that this was a structural change caused by hydrolyzed acidic water

consistent with the detachment of the cell wall from the outer layer caused by the ethanol treatment in the above report.

Micrococcus luteus is part of the normal flora of normal skin and is an environmental contaminant in soil and water (19). It is normally nonpathogenic, but its involvement in opportunistic infections, such as endocarditis, has been reported (21, 22). Figures 5B and 5D show the bactericidal effect of electrolyzed acidic water on *M. luteus* cells on electron microscopy. In Figs. 5A and 5B, *Micrococcus luteus* was embedded, thin-sectioned, and observed under an electron microscope. The cell wall, 70S type ribosomal granules, DNA in the center, and the septum were observed (Fig. 5A). The transmission (Fig. 5B) and scanning (Fig. 5D) electron microscopic findings of *M. luteus* treated with electrolyzed acidic water are presented. As seen in Fig. 5B, cytoplasmic structures such as ribosomes were lost. The electron microscopic findings indicate that cytoplasmic substances and ribosomes were exposed to electrolyzed acidic water at specific sites around the cell. On scanning electron microscopy of these cells, the cell wall was wavy and depressed, showing marked deformity of the cell morphology (Fig. 5D). Tanaka et al. (12) treated *M. luteus* cells with ethanol, and found that the number of high electron-dense granular structures increased in the cytoplasm. A similar accumulation of granular structures caused by an imidazole antifungal agent, raconazole, was observed in *Trichophyton rubrum* fungal cells by electron microscopy. However, such a cytoplasmic accumulation of granular structures was not observed in *M. luteus* treated with electrolyzed acidic water.

Some microorganisms induce various diseases, such as infectious and allergic diseases, in humans. Many cases of infections caused by bacteria with a low toxicity, considered to be nonpathogenic and to cause no disease in healthy individuals, in patients with a weak immune system have recently been reported. The numbers of such opportunistic infections have been increasing with advancements in living environments of humans and medical care. There are various educational programs in sport education universities with regard to public health and epidemiology. Information on the microbial contamination of educational environments and facilities is very important for education and the prevention of epidemics. To obtain such information, routine isolation and identification of bacteria from various sport education facilities and sport equipment are necessary, and adequate preventive measures against epidemics should be established. Electron microscopy showed that electrolyzed acidic water exhibited a bactericidal effect on the three bacterial species frequently isolated from protective kendo equipment. The analysis of the bactericidal mechanism of electrolyzed acidic water on the three bacterial species is an important subject to be investigated in the future.

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