[Environmental Health and Preventive Medicine 12, 11–16, January 2007]

Original Article

Analysis of Bacterial Flora in Dohyo Soil

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Abstract

Objectives: Sumo wrestling is one of the most popular sports in Japan. Injuries are not uncommon as this is a vigorous contact sport. Sumo wrestlers have little in the way of protective clothing; their main garb is the mawashi, making them prone to exposure to any microorganisms in the dohyo. The bacterial flora of the dohyo has received little attention. If the constituent flora is identified, then appropriate treatment or prevention of any bacterial lesions or infections incurred by the wrestlers is possible.

Methods: The Vitek AMS system used in this study was developed by McDonnell Douglas Corporation. In this system, the physiological and biochemical properties of Gram-positive and -negative bacilli, Gram-positive and -negative cocci, and fungi isolated from clinical materials and environments are examined using test cards specifically for each microorganism group, and the results are automatically read by a computer and encoded. Obtained codes are compared with a built-in database, and bacterial species of test strains are identified.

Results: In this study, using the automatic identification kit VITEK or ATB, we describe the aerobic bacterial flora found in the dohyo over the four seasons of the year. We also investigated the effect of salt on the bacterial flora as sumo wrestlers toss salt on the dohyo before each match. We show the relationship between salinity changes and variations in the flora observed upon the addition of salt. Without salt, at the beginning of a match, Gram-negative bacteria predominate. When salt is added, there is a transient decrease in the incidence of flora followed by an increase in the incidence Gram-positive cocci.

Conclusions: Sixteen bacterial genera were identified using the bacterial identification systems in dohyo soil samples during the year. The number of identified bacterial species was 32. Even in the presence of salt, there is a measurable amount of bacterial flora in dohyo soil; salt does not act as an antibacterial agent.

Key words: aerobic bacterial flora, identification, sumo wrestler, salt tossed on dohyo

Introduction

Despite the importance of promoting safety in sports, there have been only a few studies on bacterial flora in sport facilities, equipment, and protectors (1). To clarify the present status of bacterial flora living in the natural environment, we have performed detailed surveys of bacteria found in educational/workplace facilities, sport equipment, sport protectors, and uniforms using the rapid automatic bacterial identification systems VITEK and Automatic Tests in Bacteriology (ATB) as high-technology systems in our department. Our previous studies clarified the presence of bacterial flora in kendo equipment, water in pools, wrestling mats, and dance floors, and evaluated the bactericidal effects of various disinfectants in terms of hygiene, public hygiene, and epidemiology (2–5).

Sumo wrestling is a vigorous contact sport using the entire body on the dohyo, and sumo wrestlers wear only “mawashi”.

Received Jun. 2, 2006/Accepted Sep. 20, 2006
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Therefore, injuries and disorders tend to occur in sumo wrestlers. Minami et al. (6) performed a survey of pathogenic bacteria in the soil of dohyos for sumo wrestling in universities to identify bacteria causing purulent prepatellar bursitis in student sumo wrestlers. For safety in sport activities, we consider it important to clarify bacterial flora derived from dohyo and take appropriate preventive measures when necessary. The purpose of this study was to clarify aerobic bacterial flora in dohyo soil for sumo wrestling using automatic bacterial identification systems throughout a year. In addition, changes in bacterial flora with changes in the concentration of salt tossed on the dohyo were investigated.

Materials and Methods

1. Collection of samples

Soil samples obtained from the dohyo of Nippon Sport Science University (the university hereafter) and those from the centre of the dohyo in the sumo stadium Kokugikan of Nihon Sumo Kyokai were used for experiments.

Collection of soil: For the isolation of aerobic bacteria, about 5 g of soil was collected from the surface of the dohyo of the university. In the Ryogoku Kokugikan, soil samples were obtained from the center of the dohyo between the first day of the New Year's Sumo Tournament to the last day of the grand sumo tournament.

2. Culture methods for bacteria

Collected soil samples were diluted with sterile physiological saline, and the bacterial solution was inoculated in normal agar (Eiken Chemical Co., Ltd.), aerobically cultured in a thermostat at 37°C for 24 hours, and further incubated at 20–25°C for 3 days. Colonies formed on the medium were counted.

The frequency of bacterium was calculated, as follows:

$$\text{frequency} = \frac{\text{number of colonies (CFUs/g) of each bacterial species}}{\text{total number of bacterial species}} \times 100$$

3. Determination of salt concentration

Standard sodium chloride solution was prepared, and the normality of silver nitrate was determined. Using this silver nitrate, chlorine concentration in extracts from soil was determined.

4. Salt concentration and bacteria count

Bacterial suspension prepared from collected soil samples was inoculated into media with different salt concentrations (0.5–15%). The subsequent procedure was similar to that for the culture of aerobic bacteria, and colonies formed on media were counted.

5. Identification using automatic systems

For aerobic bacteria, colonies obtained from the agar medium were isolated and purified. After Gram staining, a sample was observed under a microscope. Subsequently, appropriate identification cards (VITEK or ATB) were selected on the basis of Gram staining. Each card was filled with the susupension of the test bacteria, and bacteria were identified using the rapid automatic bacteria identification systems (VITEK and ATB) (7, 8).

Results

I. Analysis of bacterial flora in dohyo soil

Figures 1a–d show bacterial species identified using the rapid automatic bacterial identification systems according to the 4 seasons. The 3-month periods from March to May, June to August, September to November, and December to February were corresponded to spring, summer, autumn, and winter, respectively. The frecuency of each bacterial species was expressed as the percentage of all bacterial species identified at 3-month intervals. The following bacterial genera were identified using the bacteria identification systems in dohyo soil samples during the year: *Acinetobacter, Bacillus, Bordetella, Escherichia, Kocuria, Micrococcus, Moraxella, Pasteurella, Plesiomonas, Proteus, Providencia, Pseudomonas, Shewanella, Sphingobacterium, Sphingomonas,* and *Staphylococcus.* The number of identified bacterial species was 32 (Figs. 1a–d). The total bacterial numbers in the four seasons were calculated by the mean of standard plate counts. The total number (4.3×10^4 CFUs/g) was highest in spring, followed by summer (3.4×10^4 CFUs/g), autumn (2.9×10^4 CFUs/g) and winter (2.3×10^4 CFUs/g) in this order.

In spring, 20 bacterial species of nine genera were isolated and identified. In the case of Gram-positive bacilli, nine species were identified: *Bacillus sphaericus, B. pumilus, B. cereus, B. subtilis, B. megaterium, B. lentus, B. thuringiensis, B. firmus,* and *B. licheniformis.* The incidences of *B. megaterium* and *B. pumilus* were 10% or higher. The incidences of *B. thuringiensis, B. cereus,* and *B. subtilis, Pseudomonas stutzeri* and *Sphingomonas paucimobilis* were 5% or more. In the case of Gram-positive cocci, *Staphylococcus hominis, S. cohnii,* and *S. xylosus* were identified, and their incidences were low. *Pasteurella multocida* and *P. haemolytica* were isolated and identified (Fig. 1a).

As Fig. 1b shows, 18 bacterial species of 10 genera were identified in summer. In the case of Gram-positive bacilli, nine species were identified in spring, and the incidences of *Bacillus sphaericus, B. cereus,* and *B. megaterium* were about 15%. *B. subtilis, B. pumilus, B. thuringiensis, B. firmus, B. licheniformis,* and *B. coagulans* were also identified. The other species frequently isolated were *Staphylococcus xylosus, Bacillus subtilis,* and *B. thuringiensis.* In addition, *Escherichia coli* as a representative intestinal bacterial species was identified (Fig. 1b).

In autumn, 13 bacterial species of five genera were isolated and identified. In the case of *Bacillus,* there were seven bacterial species: *B. megaterium, B. sphaericus, B. cereus, B. subtilis, B. pumilus,* and *B. licheniformis.* As shown in Fig. 1c, the incidence of *B. megaterium* was about 20%. Pathogenic bacteria were *Staphylococcus aureus, Bacillus cereus,* and *Pasteurella multochida,* and their incidences were about 5%. In addition, *Micrococcus lylae, M. roseus,* and *Sphingomonas paucimobilis* were also detected.

In winter, 15 bacterial species of eight genera were iso-

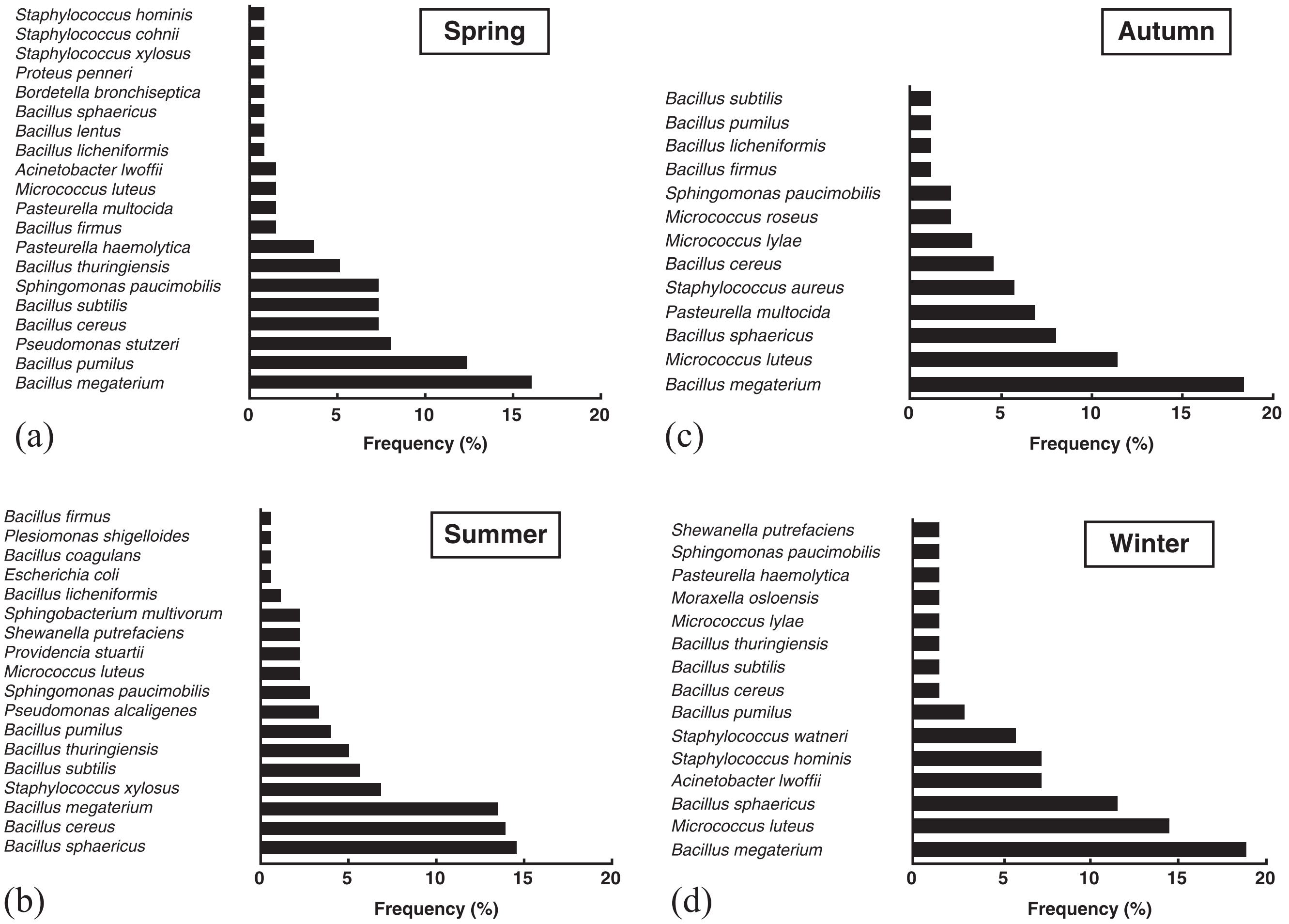

Fig. 1 a–d: Bacterial strains were detected from dohyo soil in university. Figures a–d show the 3-month periods from March to May, June to August, September to November, and December to February were corresponding to as spring, summer, autumn, and winter, respectively. Each bacterial culture was Gram stained, and then cell morphology was observed. After initial classification, the bacteria were identified using VITEK AMS and ATB automatic bacterial test systems. See text for explanation. The frequency of bacterium was calculated from number of each bacterium and total number of bacteria species.

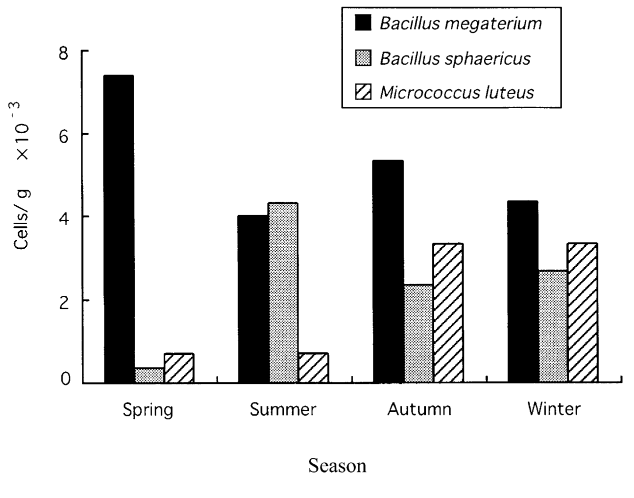

Fig. 2 Seasonal dynamics of the frequently isolated bacteria species. Changes in the frequencies of the three most frequently isolated species, namely, *Bacillus megaterium, Bacillus sphaericus,* and *Micrococcus luteus,* during the year.

lated and identified (Fig. 1d). In the case of *Bacillus,* six bacterial species were identified: *B. megaterium, B. sphaericus, B. cereus, B. subtilis, B. pumilus,* and *B. thuringiensis.* The incidence of *Bacillus megaterium* was high in winter as well as in autumn. The incidences of *Micrococcus luteus, B. sphaericus, Acinetobacter iwoffii, Staphylococcus hominis*, and *S. warneri* were high. *Pasteurella haemolytica* was also identified.

Figure 2 shows changes in the incidences of *Bacillus megaterium, B. sphaericus,* and *Micrococcus luteus* during the year among the frequently isolated bacterial species. The incidence of *Bacillus megaterium* was about 15% throughout the year (Figs. 1a–d). As shown in Fig. 2, the incidence of *Bacillus megaterium* was almost constant throughout the year, suggesting that *B. megaterium* is a representative species in dohyo soil. The incidence of *Bacillus sphaericus* was low in spring but linearly increased, reaching the peak in summer (about 15%), and decreased in autumn and winter (Fig. 2). This curve showing a linear increase and a linear decrease suggests an association between changes in the incidences of *Bacillus sphaericus* during the year and temperature. The incidence of *Micrococcus luteus* was only about 3% in spring and summer and increased in autumn and winter.

II. Correlation between salt concentration in dohyo soil and bacterial flora

In sumo wrestling, sumo wrestlers toss salt on the dohyo before each match. Therefore, salt concentration increases with the progress of matches. Bacterial proliferation is affected by salt concentration. On the dohyo of the sumo stadium Ryogoku Kokugikan of the Nihon Sumo Kyokai, the salt concentration during tournaments is high. Using dohyo soil in the Kokugikan, we evaluated changes in the salt concentration and bacterial flora during the 15-day New Year's Sumo Tournament. Table 1 shows serial changes in the salt concentration and bacterial count from before the start of the tournament to its last day in Kokugikan. Before the New Year's Tournament, the salt concentration in the soil was 0.006% (Table 1), and the count of indigenous bacteria was about 10^4 cells/g. Immediately after the start of the tournament, the salt concentration increased, and the mean salt concentration on the dohyo surface was about 35%. With an increase in the salt concentration on the dohyo, the bacterial count transiently decreased early after the initiation of the tournament but subsequently increased again (Table 2). Table 1 shows only slight changes in the bacterial count with changes in the salt concentration in the dohyo soil in the Kokugikan. Table 2 shows the results of Gram staining of bacteria isolated before the initiation of and during the New Year's Tournament. At a salt concentration of 0.006% on the dohyo, Gram-negative bacilli accounted for 92% of all isolated bacteria. The frequently detected bacterial species that were identified using the automatic bacterial identification systems were *Pseudomonas fluorescens, Escherichia coli, Xanthononas campestris,* and *Comamonas acidovorans*. The salt concentration on the dohyo was about 35% from the 8th day to the last day (15th day) of the tournament. As Table 2 shows, the percentage of Gram-positive bacilli increased threefold from 8% before the initiation of the tournament to 23% on the 8th day and to 64% on 15th day. Spore staining showed that *Bacillus* with spores constituted most of these bacteria. In addition, the amount of Gram-positive *Staphylococcus* cocci rapidly increased in the high-salt concentration environment. Therefore, after tossing salt on the dohyo, the proliferation of Gram-negative bacilli was inhibited, while Gram-positive cocci and bacilli proliferated.

Table 1 Bacterial numbers and salt concentration on dohyo during New Year's Sumo Tournament in the Ryogoku Kokugikan

Day	−1	4	8	12	15
No. of Colonies (CFUs/g)	7.7×10^4	2.3×10^4	3.8×10^4	5.4×10^4	5.3×10^4
NaCl (%)	0.006	47	36	38	32

Soil samples were obtained from the center of the dohyo between the first day of the New Year's Sumo Tournament to the last day of the grand sumo tournament. The first day (−1 day) is before the start of the 15-day sumo tournament.

Table 2 Changes in bacterial flora on the dohyo during the New Year's Sumo Tournament in Kokugikan

Bacteria	Day		
	−1	8	15
Gram-positive bacilli	8%	23%	64%
Gram-negative bacilli	92%	12%	10%
Gram-positive cocci	0%	65%	26%
Gram-negative cocci	0%	0%	0%

Note that the salt concentration on the dohyo of the Kokugikan during sumo tournaments is high. The mean salt concentration on the dohyo surface was about 35% during the sumo tournament. The first day (−1 day) is before the start of the 15-day sumo tournaments.

Discussion

For the diagnosis of disease, the identification of causative microorganisms such as bacteria is important and is widely performed in the medical clinical examination field. At present, there are some commercially available automated systems for microorganism identification. The Vitek AMS system used in this study was developed by McDonnell Douglas Corporation (U.S.) (7, 8). Using this rapid automatic bacterial identification system, many bacterial species can be identified simultaneously, and the determination time is short.

Despite the advances in medicine, the incidence of opportunistic infections has increased. We consider it very important in terms of education and disease prevention to understand the present status of bacterial flora in the living and natural environment. It may be important for sport instructors and players to understand the present status of contamination in their own workplace environment and competition facilities, not only as mere scientific knowledge but as basic knowledge for safety in sport competitions.

It was reported that pathogenic bacteria in the soil of the dohyo for sumo wrestling to identify the microorganisms causing purulent prepatellar bursitis in student sumo players (6). They reported that Gram-negative bacilli, glucose-nonfermenting Gram-negative bacilli and coagulase-negative staphylococci were isolated from the soil of the dohyo in 10 universities (6). Bacteria causing purulent prepatellar bursitis were not found in the soil of the dohyo. On the basis of these results, they suggested that salt on the dohyo has bactericidal effects on pathogenic bacteria, therefore, the dohyo environment maintains its safety because of the presence of salt (6). On the other hand, we isolated *Staphylococcus aureus* (about 6 % of all isolated microorganisms in autumn, Fig. 1c) causing purulent diseases and food poisoning from the soil of the dohyo. The purpose of our study was to clarify aerobic bacterial flora in dohyo soil during the year. There have been no studies that evaluated bacterial flora in the soil of the dohyo for sumo including that in the Kokugikan throughout the year. This study was the first to investigate bacterial flora in dohyo soil using automatic bacteria identification systems. We also evaluated serial changes in salt concentration and bacterial flora in dohyo soil. The soil of the dohyo for sumo is prepared by humans. Soil in the dohyo is frequently gathered at the center of the dohyo and evenly spread over the dohyo surface for constant leveling. Since the soil is also stirred and leveled after each sumo match, bacterial species on the dohyo surface had been equally mixed in this study. On the basis of these results, we decided to collect soil samples from the center of the dohyo throughout the year in this study. The salt concentrations in the dohyo of the university ranged from 0.4 to 1.7% throughout the year. The counts of aerobic bacteria in soil collected from the dohyo in this environment were from 2.3 to 4.3×10^4 cells/g. The 32 bacterial species were isolated and identified during the year including *Staphylococcus aureus* as shown in Fig. 1. *Bacillus* was most frequently isolated, accounting for 36–56% of all bacterial counts.

As shown in Figs. 1a–d, and 2, *Bacillus megaterium* was most frequently identified throughout the year and is considered to be a "bacterial species on the dohyo". Nine other species of *Bacillus* were isolated. Many *Bacillus* species are nonpathogenic and are widely distributed in nature, forming resistant spores. They can tolerate severe environments and are highly resistant to disinfection, dryness, and heat. The high incidence of *Bacillus* may reflect the characteristics of species of this genus. As Figs. 1a–d show, *Bacillus cereus* was isolated and identified throughout the year. *Bacillus cereus* has been reported to cause opportunistic infections such as sepsis, bronchial pneumonia, meningitis, and panophthalmitis (9).

In the case of *Staphylococcus,* five species of *Staphylococcus* causing purulent disease or food poisoning were isolated. As shown in Fig. 1c, the incidence of *Staphylococcus aureus* was 5% of all the bacterial species isolatd in this study. In summer, *Escherichia coli,* a representative intestinal bacterium was isolated. *Pasteurella haemolytica* and *P. multocida* were also isolated. In autumn, *Pasteurella multocida*, which is considered to cause zoonotic infections such as acute phlegmon, sepsis, and respiratory infection, was detected (Fig. 1c).

Tables 1 and 2 show the salt concentrations and bacteria counts during the New Year's Tournament in Kokugikan. The salt concentration in the soil increased immediately after the initiation of the tournament to about 35% (Table 1). With an increase in the salt concentration on the dohyo, the bacterial count in the soil transiently decreased but subsequently increased to about 10^4–10^5 cells/g, the same as the previous value. As Table 2 shows, the count of Gram-negative bacilli decreased with an increase in the salt concentration, whereas the Gram-positive bacilli increased, and the total bacteria count was maintained at about 10^4–10^5 cells/g. We identified bacteria using the Vitek AMS system after culture of the dohyo soil suspension before the New Year's Tournament in Kokugikan at salt concentrations adjusted to 0.5%, 5%, and 15% in normal agar medium (data not shown). Seventeen bacterial species were identified in the dohyo soil at the salt concentration of 0.5% in the medium: *Bacillus sphaericus, Bacillus megaterium, Staphylococcus kloosii, Bacillus cereus, Bacillus pumilus, Bacillus firmus, Staphylococcus warneri, Bacillus thuringiensis, Bacillus circulans, Bacillus lentus, Staphylococcus chromogenes, Staphylococcus xylosus, Staphylococcus epidermidis, Micrococcus lylae, Sphingobacterium multivorum, Sphingobacterium spiritivorum,* and *Moraxella osloensis.* At the salt concentration of 5%, eight bacterial species were identified: *Bacillus megaterium, B. firmus, Micrococcus luteus, Bacillus cereus, B. subtilis, B. sphaericus, Staphylococcus epidermidis,* and *Xanthomonas campestris*. Three species of *Staphylococcus* were identified at the salt concentration of 15%: *Staphylococcus xylosus, Staphylococcus simulans,* and *Staphylococcus kloosii.* These results suggest that the bacterial strains will tolerate and survive at these very high salt concentrations. This study showed that the proliferation of Gram-negative bacteria was inhibited with an increase in the salt concentration in the dohyo soil, whereas Gram-positive cocci proliferated instead, maintaining the bacteria count in the dohyo. We also performed a questionnaire survey on their perception on salt tossed onto the dohyo of 50 sumo wrestlers, who were professional or belonged to the sumo clubs of universities. Concerning items associated with bacteria, 67% of the respondents considered that salt tossed onto the dohyo has bactericidal effects on pathogenic bacteria on the dohyo and maintains the safety of the dohyo.

In this study, bacteria in the soil of the dohyos were isolated and identified. As a result, 10 species of *Bacillus* were identified, and the incidence of *Bacillus megaterium* was highest (about 15%) in each season. Therefore, *Bacillus megaterium* can be called "a bacterium on the dohyo". In the case of *Staphylococcus, Staphylococcus aureus* (10, 11) causing purulent diseases and food poisoning was isolated. Throughout the year, *Pseudomonas, Proteus, Shewanella, Acinetobacter, Micrococcus, Sphingobacterium,* and *Pasteurella* were observed. In summer, *Escherichia coli*, a typical bacterium in the intestinal flora was identified. Most bacterial species isolated and identified in this study were indigenous bacteria that spontaneously occur in the natural environments, originally composed the indigenous bacterial flora, and are non-pathogenic in humans. However, as described above, due to progress in advanced medicine and changes in the living environment, the incidence of opportunistic infection has been increasing. Bacteria normally that do not cause disease in healthy people sometimes cause infection. Therefore, for the appropriate management of sport facilities, equipment and proteictores, instructors and students should understand the status of bacterial contamination under normal conditions, and instructors should have basic bacteriological knowledge. In the future, studies on the sterilization of dohyo and disinfection effects will be necessary.

Reports of anaerobic bacterial flora in dohyo soil samples have apparently not been up to date. In the present study, evaluation of anaerobic bacteria in dohyo soil was excluded because of difficult methodologies of their isolation. Further investigation is necessary for analysis of anaerobic bacterial flora.

Acknowledgements

This work was supported by the Grant in Aid for Scientific Research (No. 15570054 to Osafune) from the Ministry of Education, Culture, Sports, Sciences and Technology, Japan. We thank the chief director of Nippon Sumo Kyokai, Mr. Toshimitsu Kitanoumi and Professor Emeritus, Takeo Tohono, Nippon Sport Science University who helped collect the soil from dohyo, and also Professor Harvard Lyman of Department of Biochemistry and Cell Biology, the State University of New York at Stony Brook, for critically reading this manuscript.

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