Decrease in the number of bacteria for nucleic acid extraction and sampling of microbiome from the environment

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Abstract

Purpose / Context - Public facilities such as homes for aged individuals and schools are considered at an increased risk for the spread of infections. To our knowledge, there has been no research on the relationship between the microbiome and human activity at such places. Therefore, we investigated the microbiome of locations housing individuals with weakened immune systems due to the spread of infection, to reveal its characteristics. We attempted to establish a method to sample microbiomes from these environments.

Methodology - For ease of sampling, we used *Lactobacillus* instead of the microbiome directly. We dropped the diluted *Lactobacillus* onto a plate and swabbed it. After DNA extraction from these bacteria, we calculated the number of bacteria by real-time PCR analysis.

Results - Results showed that approximately 50% of bacteria were lost during sampling and nucleic acid extraction. In this experiment, we found a faint band for the negative control by gel electrophoresis. Prevention and control of microbial contamination are required in the future.

Key Findings - Slightly fewer than 50% of initial bacteria were scattered in the air or deposited on the plate, even considering the number of bacteria after wiping.

Originality - Identification of bacteria directly and rapid analysis.

Keywords - Microbiome; real time PCR; Lactobacillus; 16S rRNA

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1. Introduction

Public facilities such as homes for aged individuals and schools are considered at high risk for spreading of infections (Rintala et al., 2008). Conventionally, transmission has been controlled by identification of the bacteria or virus from the pathogenic substance and isolation of the source of infection. To identify bacteria, colonies from cultured samples are used. However, some bacteria cannot be identified owing to culturing difficulties. Moreover, viruses cannot be cultured; therefore, we must analyze cells infected with the virus. Therefore, we cannot directly and rapidly identify and analyze viruses. However, identification and analysis speed has been recently improved with developments in gene analysis technology. (Hattori et al., 2009; Shokralla et al., 2012)

This experiment was performed to establish a microbiome sampling method based on genome analysis for fungi, bacteria, and viruses to evaluate infection risk. We could not clarify the variables for each condition (such as exposure time of sample, number of times of wiping, type of swab); therefore, we used fermented milk in this study, since one reason for the low success rate during collection was the presence of spore-forming *Lactobacillus*.

2. Methodology

First, coated wood plates of 10 cm^2 were vertically and horizontally wiped twice with 70 % alcohol-impregnated degreased cotton. Subsequently, the plate was irradiated with ultraviolet rays for 10 minutes in a safety cabinet. At this time, the researcher wiped the plate to confirm the absence of bacteria.

Fermented milk drink was diluted 100-fold, and the researcher dropped the diluted sample (0.5 μL) onto the plate at regular intervals for a total of 10 μL. The sample was then spread uniformly over the entire plate with a bacterial spreader.

Two types of swabs were used: wet and dry. The researcher pressed the swab against the wood plate with a force of 0.15 kgf and subsequently wiped the plate. Wiping proceeded vertically and horizontally 33 times, as the width of the swab was 3.0 mm. This process was repeated three times. Subsequently, we extracted DNA from 10 μl of diluted fermented milk to confirm the initial inoculum of *Lactobacillus*. Furthermore, we added 10 μl of diluted fermented milk to the swab to determine the number of *Lactobacillus* that remained on the swab.

We analyzed all samples by real-time PCR.

Table 1: Real-time PCR reaction composition (20 μl)

SYBR Fast qPCR MIX	10 μl
Forward Primer	0.1 μl
Reverse Primer	0.1 μl
Sterile Water	8.8 μl
Sample	1 μl

Table 2: PCR protocol

3 Step PCR (Cycles: 30)	1	94 °C	3 min
	2	94 °C	5 sec
	3	55 °C	1 sec
	4	68 °C	4 sec
	5	GOTO 2, 29 times	

3. Results and discussion

Figure 1 shows the standard curve from real-time PCR analysis. Correlation coefficients and amplification efficiency were within the proper range.

Table 3 shows the number of *Lactobacillus* in each sample.

Comparing Exp 1 and 2, the number of Lactobacillus decreased in the latter because the contact load was unstable. The proportion of *Lactobacillus* that remained in the swab was approximately 13 % and the proportion that attached to the bacterial spreader was approximately 5 %. Based on these results, we calculated the rate of bacteria collection.

Figure 2 shows that slightly fewer than 50% of bacteria were scattered in the air or deposited on the plate, even considering the number of bacteria after wiping.

We will next study the actual phenomenon of movement of bacteria or viruses within oral, hand, desk, and air samples.

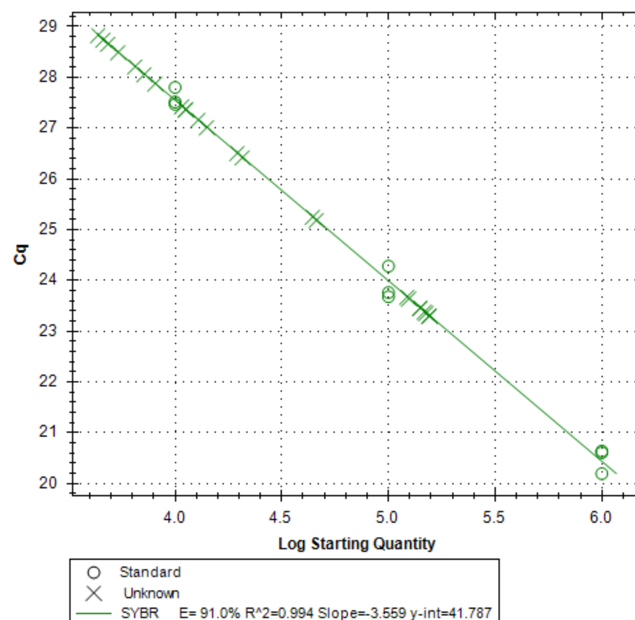

Figure 1: Standard curve chart

Table 3: Number of *Lactobacillus*

(SQ/1 µl)	First	Second	Third	Standard	Dropped Swab	Bacterial Spreader
Exp 1	45,175	11,250	4,493	155,950	141,200	5,145
Exp 2	20,180	13,490	6,851	149,400	124,000	9,448

Table 4: Sample details

Sample Name	Source
First	First-time wiping
Second	Second-time wiping
Third	Third-time wiping
Standard	Initial number in 10 µl of diluted fermented milk
Dropped Swab	10 µl of diluted fermented milk added directly to swab
Bacterial Spreader	*Lactobacillus* attached to bacterial spreader

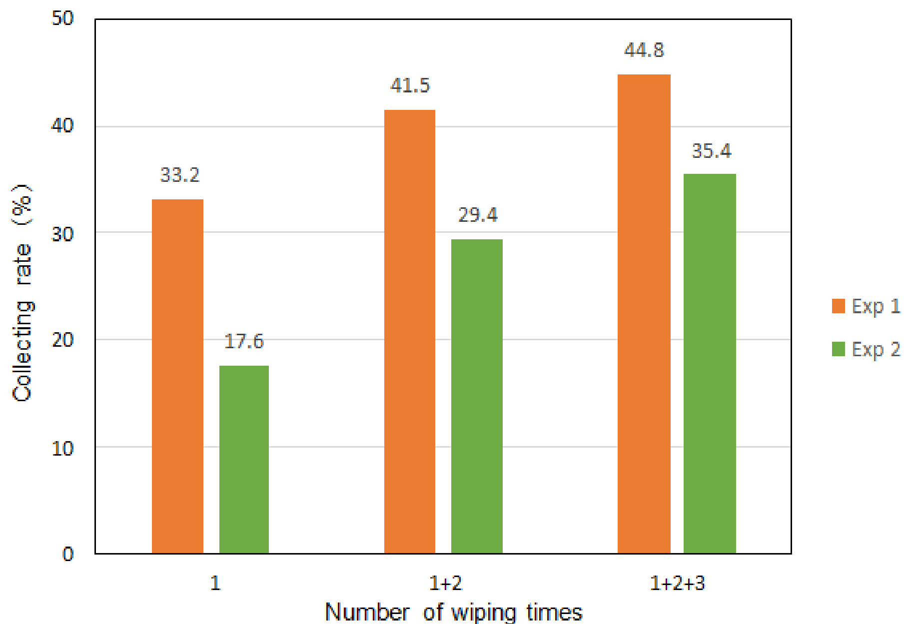

Figure 2: Collection rate of *Lactobacillus*

4. Conclusion

This experiment was performed to establish a sampling method based on genome analysis for microbiomes that are composed of fungi, bacteria, and viruses, to evaluate the risk of infection. The results indicate the following:

(1) Slightly fewer than 50% of the bacteria were scattered in the air or deposited on the plate, even when considering the number of bacteria after wiping.
(2) If the number of bacteria obtained after wiping three times was the total number of bacteria adhering to the plate, we can obtain more than 80% of bacteria by wiping two times.

In addition, future tasks are needed as follows:

(1) To determine the cause of contamination and to reliably prevent contamination during the experiment. Due to contamination, this method would not be able to sample a small number of bacteria, as it was not possible to obtain accurate data.
(2) To perform this experiment with simultaneous air sampling to assess the number of bacteria scattered in the air. This is based on the possibility that bacteria were scattered in the air with drying and upon dropping *Lactobacillus* on the plate.
(3) To consider differences in number of bacteria collected due to the contact load. This is based on the possibility that the contact load led to a reduction in the number of bacteria harvested, because it was not constant.

5. References

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