Fundamental Study on the Rapid Detection Method of Indoor Airborne Microbes and its Applicability to Food Plants for Quality and Safety Improvement

Ze Liu\(^*\), Takehiro Tanaka\(^1\)
\(^1\)Toyo University, Saitama, 350-8585, Japan
*Corresponding author’s e-mail: hiya3506@yahoo.co.jp
Author’s e-mail: tanaka@toyo.jp

Abstract. Airborne microbes are examined generally by sampling them on culture media and counting the number of their colonies formed. However, because it takes at least two days for bacteria to be cultivated and five days for fungi, reliable results may not be obtained for a time of need. Therefore, the development of a simple and quick detection method is required. A rapid detection method of indoor airborne microbes is examined and a fundamental study on its applicability to food plants for the purpose of improving quality and safety is introduced in this study.

1. Introduction
Generally, airborne microbes are examined by sampling them on culture media and counting the number of their colonies formed. However, because it takes at least two days for bacteria to be cultivated and five days for fungi, reliable results may not be obtained for a time of need. Therefore, the development of a simple and quick detection method is required.

The second chapter introduces the current rapid detection method of microorganisms, and focuses on the ATP detection method.

The third chapter focuses on the possibility of fluorescent staining.

The fourth chapter focuses on the possibility of introducing rapid microbiological testing methods into food factories based on the HACCP benchmark.

A rapid detection method of indoor airborne microbes is examined and a fundamental study on its applicability to food plants for the purpose of improving quality and safety is introduced in this study.

2. Rapid detection methods
Rapid detection methods are roughly classified into direct methods or indirect methods. Direct methods include solid-phase cytometry and flow cytometry. In indirect methods, bacterial properties such as antigen, diffusion, adenosine tri-phosphate (ATP), proliferation potency, bacillary constituent, and deoxyribo nucleic acid (DNA) are extracted.

In many food plants, swiftness is regarded important. This study suggests the membrane filter technique be used to collect indoor airborne bacteria and the ATP assay (ATP swab test) to confirm cleanliness.

By way of comparison with the ATP assay result, the fluorescent staining technique is demonstrated although it is not regarded as a rapid measuring method.
2.1. Sampling of indoor airborne bacteria
In this study, the membrane filter technique using a pump and suction filtration system was employed to collect indoor airborne bacteria. The membrane filter technique is suitable for collecting microbes and particulates because its porous film filter has a pore structure characterized by a number of uniform circular pores connecting each other. Naturally, it is crucial to select a pore size appropriate for the collection of indoor airborne bacteria in a food plant.

2.2. ATP assay to verify cleanliness
The ATP-based testing for assessing cleanliness has been receiving much attention. Applied partially of fully, the method has become much popular, because only ten or so seconds is required to obtain a result with the ATP assay while more than 24 hours are spent in conventional culture methods. Thus, the ATP assay has been highly esteemed as a rapid sanitary inspection tool for the judgment of cleanliness and hygiene after washing in food plants and medical fields where hygiene management is highly prioritized.

The ATP assay is applicable not only to microbes but to all biological substances (organic matters). If biological contamination occurs in a food plant, bacteriological inspection alone is not sufficient but it is inevitable to carry out the ATP assay to prevent microbes from proliferating.

The conventional sterilization process works effective against surface microbes. But if residues still remain after washing, microbes within the residues survive and proliferate by taking nourishment from the residues. The above mentioned problem tends to be passed over by the stamp method as it deals with surface germs. In the bacteriological culture test, it takes more than 24 hours after cultivation to obtain a result, which sometimes makes it difficult for a researcher to devise appropriate countermeasures. The ATP assay for biological substances, which detects biological contamination easily and promptly on the spot, has a merit in that corrective measures such as re-washing can be carried out then and there. This inspection method is mentioned in “Standard Methods of Analysis in Food Safety Regulation for Microorganisms” compiled under the Ministry of Health, Labour and Welfare. In the field of space development, the ATP assay is used to see whether living things exist or not in a planet.

2.3. ATP
ATP is the abbreviation for “Adenosine Tri-Phosphate,” which is an energy-carrying molecule found in the cells of any plants, animals and microbes, exhibiting a molecular structure shown in Figure 1. ATP is a chemical compound used by all living things to provide energy in many metabolic processes: e.g. cell proliferation, muscle contraction, photosynthesis by plants, bacterial respiration, and yeast fermentation. ATP is contained in all organic matters (living things and their traces) including foods, bacteria, molds and other microbes. In this context, ATP detected on the surface and in the washing water suggests the presence of biological substances (e.g. food residue) which may promote contamination or proliferation of microbes invisible to the naked eye.

![Figure 1. Molecular structure of ATP.](image)

2.4. Principle of ATP measurement.
The principle of ATP measurement is based on the mechanism of firefly bioluminescence as shown in Figure 2. which makes it possible to measure an extremely small quantity of ATP. In the ATP assay,
specific reagents are applied to ATP samples swabbed off or contained in water to let the following chemical reaction occur and the amount of luminescence produced in the reaction is measured.

\[
\text{ATP + luciferin + enzyme} \\
\text{Mg}^{2+} \\
\text{AMP + pyrophosphoric acid + oxyluciferin + carbon dioxide + light}
\]

Figure 2. Principle of ATP measurement.

In 2009, Kikkoman Biochemifa Company developed “Lumitester” which enables researchers to conduct more precise measurements by measuring not only ATP but also Adenosine Monophosphate (AMP), a derivative of ATP heated and transformed (Patent No.3409962) [1].

3. Fluorescent staining technique
To compare with the ATP assay result, the fluorescent staining technique is demonstrated in this study although it is not regarded as a rapid measuring method. The fluorescent staining technique studied by Nasu et al. was developed in the 1970s, which has been implemented since 1990s but little applied to studies on indoor environments. It is necessary to select stains effective to the measurement and establish a method for analyzing the concentration of indoor airborne bacteria. The fluorescent staining technique demonstrated in a laboratory trial on the supposition of a food plant is shown in Photo-2. Although in the trial stage at this point for the purpose of comparing with the ATP assay result, this method is mostly completed. Therefore, it is necessary to examine a detailed evaluation method and its application to food plants.

4. Application to food plants under the HACCP system
HACCP is the acronym for “Hazard Analysis and Critical Control Point” which is a management system to secure safety in food production. In this system, damage is preliminarily prevented by predicting and analyzing the possible occurrence of adulteration hazards at specific stages in the process of manufacturing and shipping foods. Based on the general hygiene management, critical control points (CCP) in each process are monitored in order to ensure food safety.

Whenever measurement is conducted with the ATP assay or the fluorescent staining technique as proposed in this study, it is inevitable to set up control points. That is to say, hazardous points within the facility where contamination or microbial proliferation can be produced by production residue need to be identified before testing. Regarding to the control point, it is important to regularly inspect not only where products touch directly but also where indirect hazard may occur and where cleaning is difficult to be given. Those issues are discussed in the “Standard Methods of Analysis in Food Safety Regulation for Microorganisms” compiled under the Ministry of Health, Labour and Welfare. Similarly, those are also mentioned in Good Manufacturing Practice (GMP) as well as International Organization for Standardization (ISO).

In terms of food contact, areas in food plants are classified into the following three domains.

4.1. Direct contact areas
The area where products are possibly exposed to a polluted surface and contaminated is considered a high risk area. If there is a surface (e.g. conveyor belt) which products directly touch, an inspection needs to be conducted at several different places to confirm overall cleanliness. Direct contact areas include conveyor belts, washing buckets, cutting boards, slicers and other cooking devices.
4.2. Indirect contact areas
In the area where food fragments or contaminated substances are scattered on products, discharged or carried away, the source of pollution is likely to be overseen. Thus, regular inspection should be carried out. Sinks, water pipes, side walls, and additional mechanical parts are some examples.

4.3. Hard-to-clean contact areas
Because the area which is difficult to be cleaned can be a hotbed of bacterial proliferation, the place needs to be regularly inspected. According to the sampling method proposed for hose nozzles, a threaded portion outside the nozzle is wiped twice with a cotton swab and then the inside edge once. To wipe off with a cotton swab, fairly firm pressure must be applied. Hoses, O-shaped rings, nozzles, complicated surfaces, corners, grooves, cracks, and joints are some examples.

5. Summary and future issues
In this study, the rapid testing method of indoor airborne microbes was examined and a fundamental study on its applicability to food plants was carried out for the purpose of improving quality and safety. As the analytical rapidity tends to be prioritized in food plants, this study suggests that the membrane filter technique is used to collect indoor airborne microbes, and the ATP assay for inspection of cleanliness. Although not regarded as a rapid measuring method, the fluorescent staining technique was used to compare with the ATP assay result. As a preliminary experiment in the next stage, the colonies formed under the fluorescent activity staining method were visually observed in the case of dark field. Since the number and size of the colonies were not visible to the naked eye, a biological microscope was introduced for further observation and research in the future. It is consideration the possibility of introducing fluorescent activity staining into food factories. The experimental picture is shown in Figure 3. As a future issue, the fluorescent staining technique performed to compare with the ATP assay result will be solidly established, and a detailed evaluation method and its application to food plants will be examined further.

Figure 3. Experimental picture using fluorescence activity staining.

References
[1] Kikkoman. AMP-based measurement technique. Japanese patent: No. 3409962.
[2] Yanagi, U. (2018). Current status of rapid microbial detection systems. Journal of Japan Air Cleaning Association, 56(1).
[3] Nasu, M. (2016). To collect environmental microbes from a viewpoint - From household to space living. Lecture and Abstract for the proceedings of the 33rd Annual Technical Meeting on Air Cleaning and Contamination Control.
[4] Kikkoman Biochemifa Company. (2018). ATP assay under the HACCP hygiene management.
[5] Fujii, S et al. (2019). Rapid Measurement of Floating Airborne Microorganism Concentration, Lecture and Abstract for the proceedings of the 36th Annual Technical Meeting on Air Cleaning and Contamination Control.