1. Effects of Bioaerosols on the Human Body

I am researching bioaerosols. Bioaerosols are microorganisms or pieces of plant and animal cells that have been swept up into the atmosphere. By staining particles of Asian dust (kusa) collected from the atmosphere above the Taklamakan Desert in China and looking at them under a fluorescence microscope I discovered that some particles contain DNA. What this means is that there were microorganisms transported by Asian dust events. If strong westerly winds all over Asia blow thousands of meters above the ground and if microorganisms are suspended above the Taklamakan Desert then it is possible that these microorganisms can float all the way to Japan. Therefore, I examined the negative effects of bioaerosols on humans, plants, animals, and marine life.

Research on bioaerosols at the global level only started seven to eight years ago and it was around that time that I too started research on bioaerosols. For that reason, at the beginning we were tasked with researching the nature and types of microorganisms suspended in the atmosphere.

Through this initial research we discovered that some suspended fungal cells were harmful, especially *Bjerkandera adusta*. We looked at allergic reactions caused by the fungus by placing finely crushed *Bjerkandera adusta* fibers and Asian dust particles on the noses of mice. Compared to mice that had only Asian dust particles placed on the noses, we discovered a 10-fold increase in the number of mucus producing goblet cells in the bronchial tubes of mice that had both Asian dust particles and *Bjerkandera adusta* fibers placed on their noses. Therefore, by adding *Bjerkandera adusta* to Asian dust particles we were able to elicit an allergic reaction that was 10 times worse. I announced these results based on a joint research with Professor Takamichi Ichinose. Since there is now no doubt that bioaerosols exacerbate allergies, it is obvious that we must be careful not only of Asian dust particles but the microorganisms contained within.

Researchers are also reporting on other various effects of bioaerosols such as the exacerbation hay fever and Asian dust allergies, the distribution of airborne microorganism allergens by wind, the transmission of Kawasaki disease, and the spread of foot and mouth disease/wheat rust contagions. From now on we must understand the composition and think about the physiology of organisms contained in wind-blown Asian dust.

After looking into the types of microorganisms in the atmosphere, I divided them into two large groups. One group includes those that produce sugars and the other group is those that break down organic matter. From this information I proposed the following three hypotheses about how these microorganisms found in the atmosphere behave. The first hypothesis is that the microorganisms propagate on the ground. The second is that the microorganisms that produce sugars create biofilms. The third is that these biofilms are broken down by the microorganisms that break down organic matter. Since biofilms are composed of organisms, they are lighter than the sand particles, and since the biofilms contain sugars, they are sticky and will adhere to the sand particles. It is for these reasons that I believe that microorganisms are able to be swept up into the atmosphere and...
are able to tolerate the conditions found there and survive.

Since it is possible that some of the microorganisms that create biofilms cause disease, it is necessary to investigate them further. Although I am currently researching at the level of 800 to 1000 meters above the ground, it is possible that some microorganisms are being ejected into space. Therefore, there is currently a JAXA project running to investigate microorganisms in the stratosphere.

2. Investigation of Bioaerosols Coming from the Chinese Mainland

We are conducting ongoing research into microorganisms coming from the Chinese mainland along with Asian dust and particulate matter smaller than 2.5 microns (PM2.5). However, if we sample air close to the ground in Japan we will also pick up microorganisms that floated up from the ground. In order to capture microorganisms that just arrived from China we decided to take air samples by using airplane to fly up to 2000 to 3000 meters where the westerly winds blow. We stuck a tube out of the airplane through a 1.5 cm diameter hole in the roof, pumped in air from the outside, and passed it through a filter to collect particles. However, renting an airplane is expensive and requires a reservation at least two weeks in advance.

That is why we are also using balloons, which are cheaper than using an airplane. We can set the balloon free, let the winds take it where it will, and easily collect air samples. The balloon is attached to a line at the bottom and we are able to increase or decrease its altitude. Attached to the bottom of the balloon are instruments that measure particles in the air, air temperature and humidity, altitude, as well as a sampler that collects bioaerosols. Once the balloon reaches an altitude of 800 to 1000 meters, we stop releasing line to keep the balloon from rising further. Using a transceiver on the ground, we then send a signal to begin pumping outside air through the sampler. We stop the pump after about an hour and close the sampler inlet shutter. After bringing the balloon back to the ground, we take the filter out of the sampler and begin our analysis.

Since the air that flows over on the westerly winds from the Chinese mainland first arrives at the Noto Peninsula, we call the Noto the ‘Sea of Japan Antenna’. We collect our samples at the tip of the Noto peninsula in Suzu City. We are also collecting samples from the snowpack of Mount Tate. The particles in the air that were carried from the mainland can be found in the snowpack. At the beginning of spring, we climb Tateyama as far as Murodo to an elevation of 2450 meters and dig to create a cross section of snow. We create a cross section by digging into the snow and cutting a flat face of snow layers that looks like an inverted pyramid cut in half. If you take a look at the snow cross section you can see places that are yellow from accumulated Asian dust as well as blackened areas that indicate accumulated PM2.5. Aerosols that fell along with the snow from autumn through spring form a stratified time series. Taking care to prevent outside contamination of microorganisms, we take back samples from these Asian dust and PM2.5 layers for analysis.

After using these methods to collect samples, the first thing that we do is create isolation
cultures of the microorganisms found within. Samples captured in filters are extracted by suspending the filters in saline solution. Samples collected from Mount Tate snow are melted at room temperature. Samples are then spread on an agar medium and begin cultivation at an appropriate temperature. Microorganisms found in the samples are initially impossible to see on the agar medium. Microorganisms that reproduce quickly divide at a rate of once every 30 minutes, whereas slower organisms divide at a rate of once per day or once per week. For this reason, by the next day or after about a week the microorganisms form visually confirmable colonies on the agar medium. Then we scrape off the microorganisms and spread them on a new agar medium. In this way we are able to separate the different strains and grow them indoors. As long as you are able to maintain this process you are able to grow enough cells to run experiments to determine the species and characteristics of the microorganisms.

Most of the microorganisms collected from the Noto Peninsula and Mount Tate were of the \textit{Bacillus} genus. Microorganisms of the \textit{Bacillus} genus are able to create membranes by producing organic matter and then break down then membranes that they created. Therefore, it is not unusual that these broken down membranes along with the \textit{Bacillus} bacteria are swept up in the air and can be found in the air. In addition, \textit{Bacillus} bacteria are able to form resilient spores if environmental conditions worsen and can survive the harsh environmental conditions found in the atmosphere. We were able to actually capture from the atmosphere a lot of \textit{Bacillus} genus bacteria that are believed to have been transported long distances from the Chinese mainland.

However, these are only samples that we took in Japan, so we had to check whether \textit{Bacillus} bacteria were also really present in China. We focused on Dunhuang on the eastern edge of the Taklamakan Desert. We collected samples at Dunhuang, the very source of the Asian dust. Dunhuang has a splendid airport and cityscape, but outside of the city is a desert expanse. We conducted our sampling mainly at Dunhuang's weather bureau. There is a large plaza in front of the weather bureau where we sent a balloon to 800 to 1000 meters to collect particles floating in the atmosphere. We also collected air samples at the ground from the roof of the weather bureau. Depending on the situation we also went out to the desert to conduct sampling. We then created isolation cultures and examined the microorganisms that we collected. Results showed that \textit{Bacillus} bacteria also exist in Dunhuang. Looking closer into the DNA of the bacteria, we found that samples included bacteria that are genetically close to the \textit{Bacillus} bacteria found in Japan. Therefore, we were able to determine that it is extremely probable that the \textit{Bacillus} bacteria found in Japan flew over from the Chinese mainland.

However, there is a problem with the analysis method, which is the use of isolation cultures. Among the microorganisms in the environment, only 1% may be analyzed using isolation cultures. This means that 99% of the microorganisms are being overlooked. Therefore, we extracted DNA directly from the collected particles without producing cultures and conducted an exhaustive analysis.
of the entirety of the DNA contained within to determine the microorganisms present in the particles. Results showed that indeed Bacillus bacteria can be seen both on the ground and in the air above the Taklamakan Desert and other groups of bacteria were also uniformly found in both the air and on the ground. Since the wind is extremely strong in deserts and blows from the ground all the way up into the atmosphere there is a uniform mixture of different microorganisms.

What is the situation in the Noto Peninsula? Although there is an increased percentage of Bacillus bacteria at 3000 meters, at lower altitudes the percentage of Bacillus bacteria decreases as the percentage of microorganisms peculiar to Japan increases. Since we were able to determine that Bacillus bacteria are found in the atmosphere without using isolation cultures the case becomes even stronger that they were transported over from China.

We then created a family tree of the different types microorganism found in the atmosphere using a more detailed analysis. We found varieties of bacteria that can cause adverse effects in humans and animals such as Bacillus cereus that causes food poisoning, normally benign Bacillus subtilis that causes blood poisoning in those in poor health, and bacteria from the Staphylococcus genus that also causes food poisoning. This means that disease-causing microorganisms can travel long distances in the atmosphere and be spread across large areas. Since I now have a number of different species of microorganisms available for examination I would like to conduct further research on their physiological characteristics.

3. Can Bioaerosols Be Useful?

My research collaborators and I have published papers and made presentations at academic meetings about how bioaerosols can spread disease-causing microorganisms. However, we have been to Dunhuang and have been conducting research together with researchers from China. I would feel sorry for the Chinese side if we only focused on the negative, and indeed there has been some grumbling from our research collaborators. Therefore, I decided to change my perspective and look at the positive aspects of these microorganisms. When I did that, just like how people have positive and negative aspects, I was able to find some positive points of atmospheric microorganisms.

Atmospheric microorganisms cause water droplets to coalesce to create clouds, but there are some microorganisms that are better at doing this than others. If we are able to understand the mechanisms by which microorganisms create clouds then in the future it may be possible to apply this knowledge towards producing agents for creating artificial rain and snow. In fact, Pseudomonas syringae bacteria are being utilized in the United States in Snomax, a snow inducer that is being used on ski slopes.

In addition, the upper layers of the atmosphere are a harsh environment for microorganisms, being arid and bombarded by ultraviolet radiation. Since microorganisms stay alive floating around in these conditions, it is thought that they are producing some kind of material that allows them to
endure the aridity and ultraviolet rays. Whether we are able to use this material to develop new beauty products or anticancer therapies is currently being looked at.

Additionally, some of these microorganisms may be used in fermentation, such as *Staphylococcus* that is used in producing fish sauce. Although you are not able to produce fish sauce only with *Staphylococcus*, there are reports that this bacterium is necessary for improving the flavor. Among the microorganisms used in producing fermented foodstuffs we should pay special attention to *Bacillus subtilis*. We found a microorganism closely related to *Bacillus subtilis* in large quantities in samples taken from the atmosphere. It would not be a good thing if this bacterium were to negatively impact health. However, you are only able to contract an illness if *Bacillus subtilis* found its way into your blood. Otherwise, it is extremely safe. After doing some research, we found many papers that mentioned that *Bacillus subtilis* may be used to produce fermented soybeans (*natto*). Indeed, according to the literature, most *Bacillus subtilis* species are capable of producing *natto*, and although the species of microorganism that we isolated from particles in the atmosphere is *Bacillus subtilis*, whether they could be used to produce *natto* is a different story altogether.

At this point, using the microorganisms that we collected from the atmosphere we decided to try making *natto* at the Kinjyo Brand factory. In order to make *natto*, first we boiled the soybeans. Next, we created a liquid containing bacteria collected from above the Noto Peninsula and sprayed it on the soybeans. The soybeans were then packaged and fermented at 40 degrees Celsius. The end result is that we were able to successfully make *natto*. Interestingly, the two different strains of Si-37 and Si-39 that were collected at the same place and time created two very different kinds of *natto*. The Si-37 strain created an extremely gooey *natto*, whereas the Si-39 strain created a *natto* that was not at all gooey despite having *natto*’s characteristic smell. Despite being of the same species, the different strains created *natto* with very different levels of gooiness. This is extremely interesting when thinking of the nature of microorganisms.

By the way, although it may seem like a sensational story that we were able to collect *Bacillus subtilis* from the sky to make *natto*, from a microbiological perspective this is quite ordinary. Why is it that *natto* bacteria float in the sky? Normally, when *natto* bacteria reproduce, they create a long and thin chain that looks like sausage links. They are called trophocytes. However, if the environment becomes too harsh they transform into spores. Once they are in their spore form they are able to withstand harsh environmental conditions and will not die even if submerged in boiling water or exposed to negative 80 degrees Celsius conditions. They can also endure quite dry conditions. Left alone, even without finding a place to thrive they can survive for one to two years. That is why they are able to float thousands of meters up in the atmosphere and continue to live. Indeed, they may keep floating around until they fall to the ground in a new location. In other words, it is easy for *natto* bacteria to survive in the sky. For this reason the bacteria of the *Bacillus* genus and *Bacillus subtilis* in particular are more able to survive on particles of Asian dust as compared to other kinds of bacteria.
Those who really like natto may be disappointed in the natto produced by using microorganisms found in the atmosphere since it contains less of the goo-creating polyglutamic acid than products normally sold in stores. That being the case, the goo has high water content and a large amount of calcium and magnesium are eluted from the soybeans. From the standpoint of the ability to break down reactive oxygen, the natto we made had the same properties as natto normally sold in stores. That is why we thought that it would be a good idea to try to market the natto that we made. On Natto Day on July 10, 2012 we started selling ‘Sora Natto’ and we are entering our fourth year of production. This natto produced using the Bacillus subtilis bacteria that we collected 3000 meters above the Noto Peninsula is unfortunately almost exclusively sold within Ishikawa Prefecture. It is being sold at the Kanazawa University student co-op cafeteria and at MaxValu, Aeon, and Albis stores within Ishikawa Prefecture. We are also happy to say that Sora Natto is being offered as a part of in-flight meals for JAL business class customers as of November 2014. If you have a chance, please try Sora Natto for yourself. You may have the realization that the natto you are eating was produced with bacteria that floats in the air.