

# バイオエアロゾル

Yasunobu IWASAKA

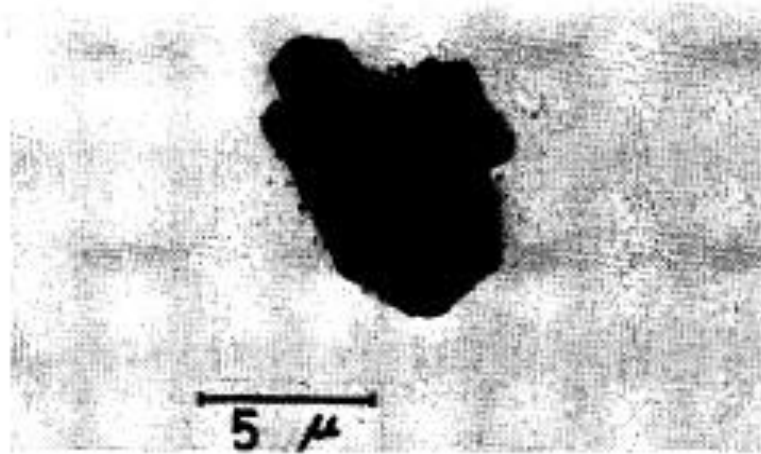
## 磯野謙治先生を偲ぶ

名古屋大学名誉教授、元日本気象学会理事長、日本気象学会名誉会員、磯野謙治先生は平成18年(2006年)1月7日、肺炎のため92歳で逝去された。昭和13年3月東京帝国大学理学部物理学科を卒業、4月1日に中央気象台に入台、ラジオゾンデの調査研究、とくに3極真空管の仕事を命ぜられた。当時3極管は普及しておらず、卒業論文でそれを扱ったからだった。15年4月1日に高層気象業務を主管する航空気象課が設置され先生は同課研究係、16年12月2日同課研究係長、17年4月1日同課技術係長、B29の空襲を受け同課が長野県に疎開するとき高層課(岩村田)と航空課(岡谷)に分かれ、先生は20年4月30日観測部高層課に配属、終戦で9月東京に戻り、21年5月3日中央気象台研究



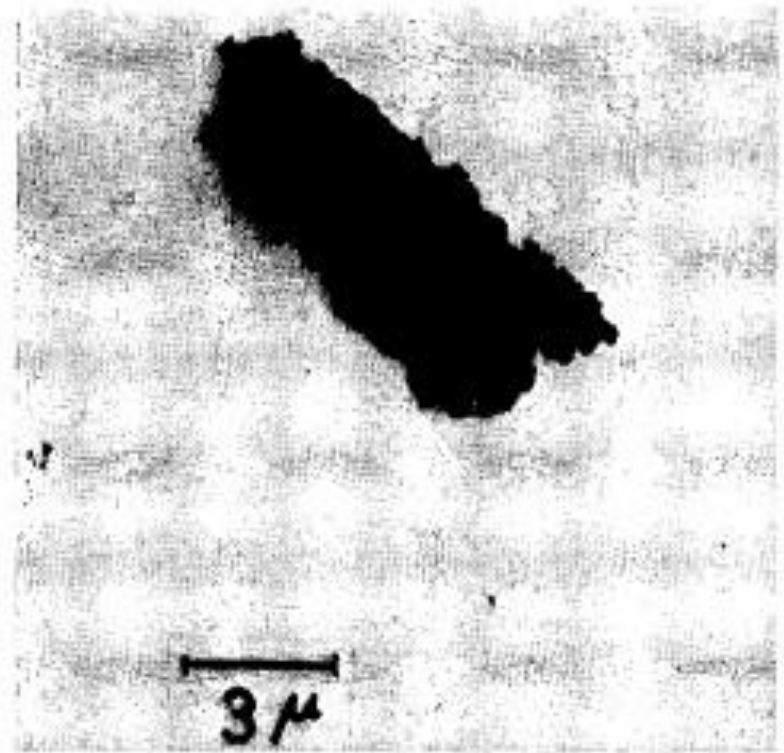
In Japan Prof. Isono firstly studied KOSA particles and discussed the mineralogical feature, and suggested the large potential as ice nuclei using electron-microscopic technique.

(Isono et. al, J. Meteor. Soc. Japan, 1959; Tellus, 1971)



*Fig. 11.* Ice nucleus on 14 February at Nagoya (kaolin mineral).

On days with low ice nucleus concentrations, particles which are considered to have originated from sea sprays are found. Figs. 15 and 16 show such particles. The shapes of the particles and the round traces or residuals of evaporated water droplets around them suggest that they acted as giant condensation nuclei and that water droplets which formed on them grew to sizes large enough to be collected by the impactor. The particles in Fig. 15*a* gave rings of NaCl as shown in Fig. 15*b*, whereas particles in Fig. 16*a* gave those of CaSO<sub>4</sub> and CaCl<sub>2</sub>. One



*Fig. 12.* Ice nucleus (clay mineral) on 25 January at Nagoya.

of the authors (Isono, 1959) found by the electron diffraction method that particles of different compositions formed when sea water was sprayed. Particles of sea salt constituents were commonly found in maritime air from the Pacific Ocean in Nagoya.

# 日本上空でつかまった 黄砂の電子顕微鏡写真

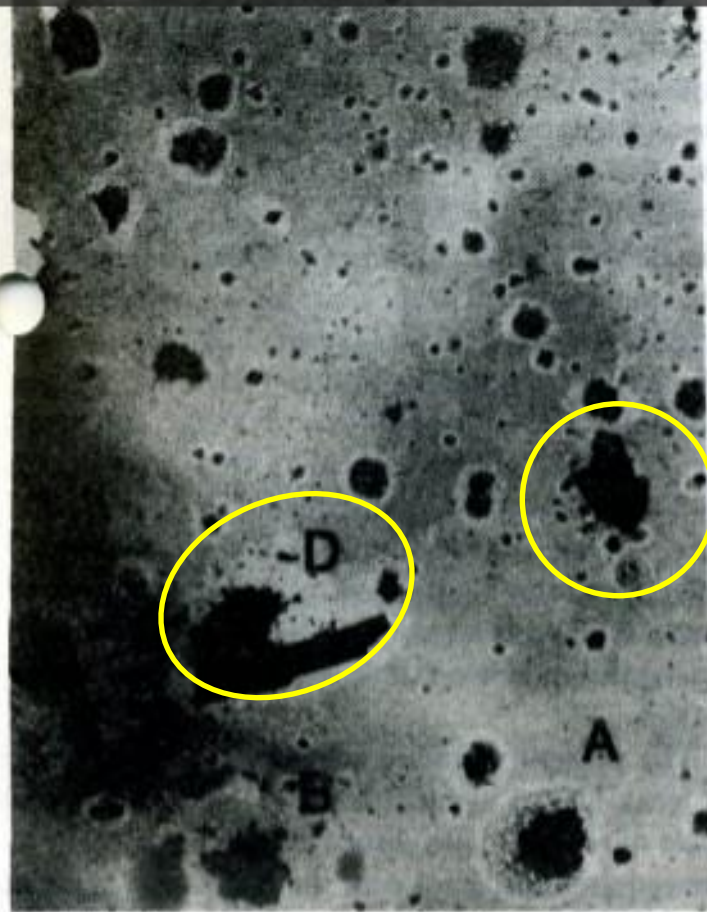
同じ黄砂の写真でも前のと大違い  
(こちらのほうは相当に弱い電子戦で観  
察している)

This picture is an important trigger to  
consider the effect of long range transport  
of Asian pollutants

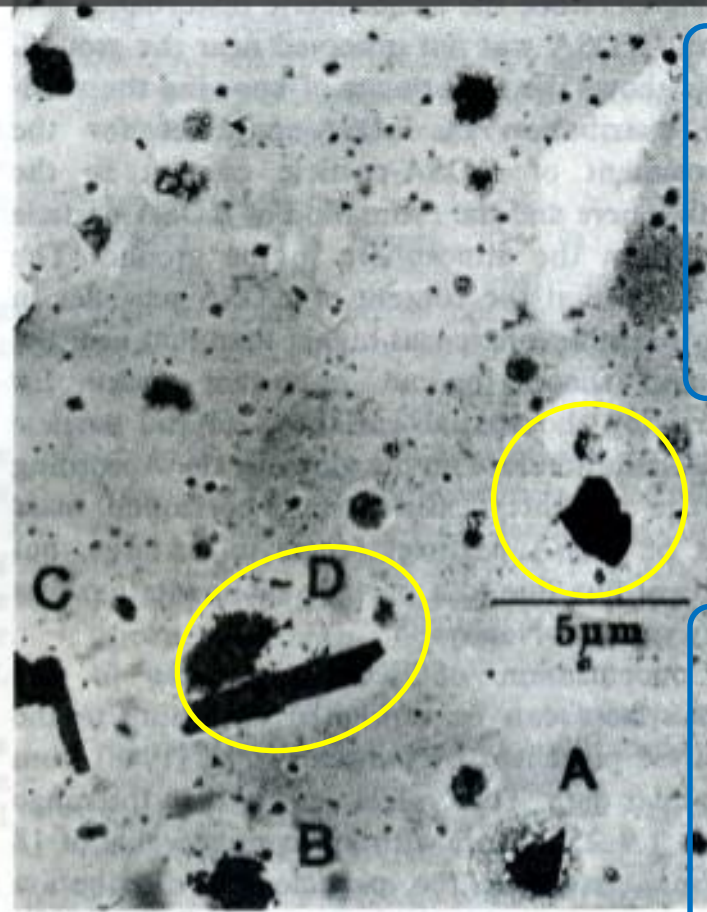
黄砂

黄砂表面の  
溶液が  
染み出した

カルシウムの膜と  
反応する硫酸！？



weak beam



strong beam

黄砂の電子顕微鏡写真。エネルギーの高い電子線を当てると表面の物が蒸発する。

黄砂の表面に何物かが載っていることもあるのでは、と思われたが当時は注目されなかった

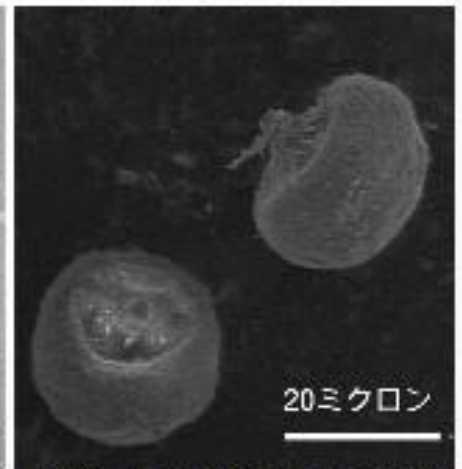
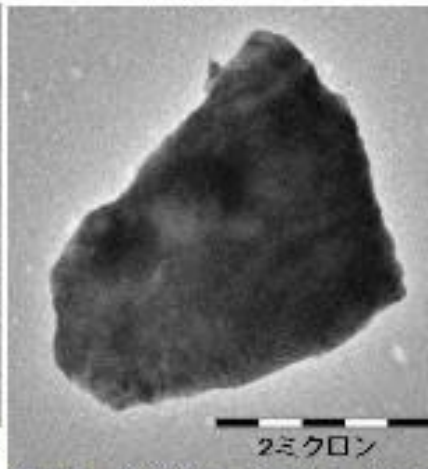
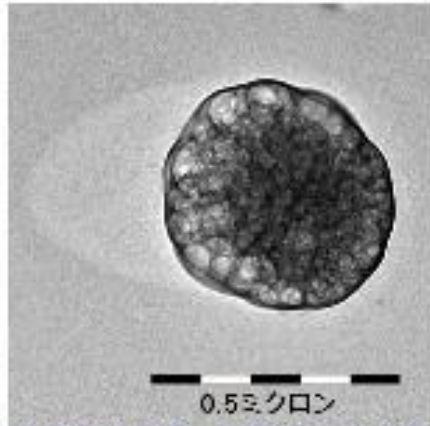
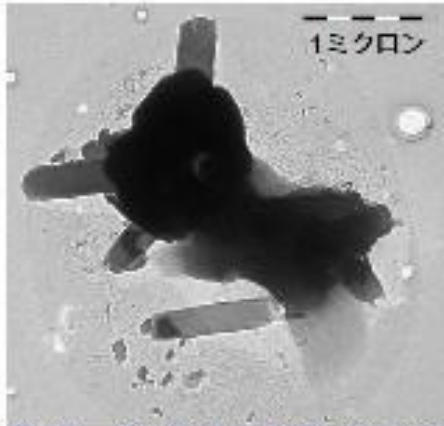
Fig. 7. KOSA particle coated solution containing  $\text{SO}_4^{2-}$  (A, B, C and D). These particles were sampled at 4350 m during the flight of 23 April, 14:00–16:00 LT. Electron micrograph under the weak electron beam (left) and that under the strong beam (right). Faint  $\text{CaSO}_4$  dots produced from Ca and  $\text{SO}_4^{2-}$  in solution on the particle surface are observed around particle A. After the radiation of the strong electron beam, unknown volatile material evaporated from the particle surface. Particles B and C were also coated with solution. However, the trace of the solution does not clearly show the existence of  $\text{SO}_4$ . The electron microgram taken under the strong electron beam showed that the particle D was composed of two different particles. This suggests that there is a possibility of coagulation between KOSA particles and other types of particle in the atmosphere.

海塩粒子

硫酸塩粒子

鉱物粒子

スギ花粉粒子



第7図 海塩粒子と硫酸塩粒子の電子顕微鏡写真。財前祐二さん提供。蓋木(2014)「雲の中では何が起きているのか」より。

第8図 鉱物粒子(アルミノシリケート)とスギ花粉粒子の電子顕微鏡写真。左は財前祐二さん、右は岩田歩さん提供蓋木(2014)「雲の中では何が起きているのか」より。

## 代表的な凝結核粒子(エアロゾル)の電子顕微鏡写真 (気象研究所のホームページより)

(微生物由来のものが出ており、面白いが、ここでスギ花粉を出すのはいかかなものか？)(電子顕微鏡写真が実態を表していると考えると大違い！！)

**今になって見て改めて  
鉍物表面にあったものは  
何であったのか？**

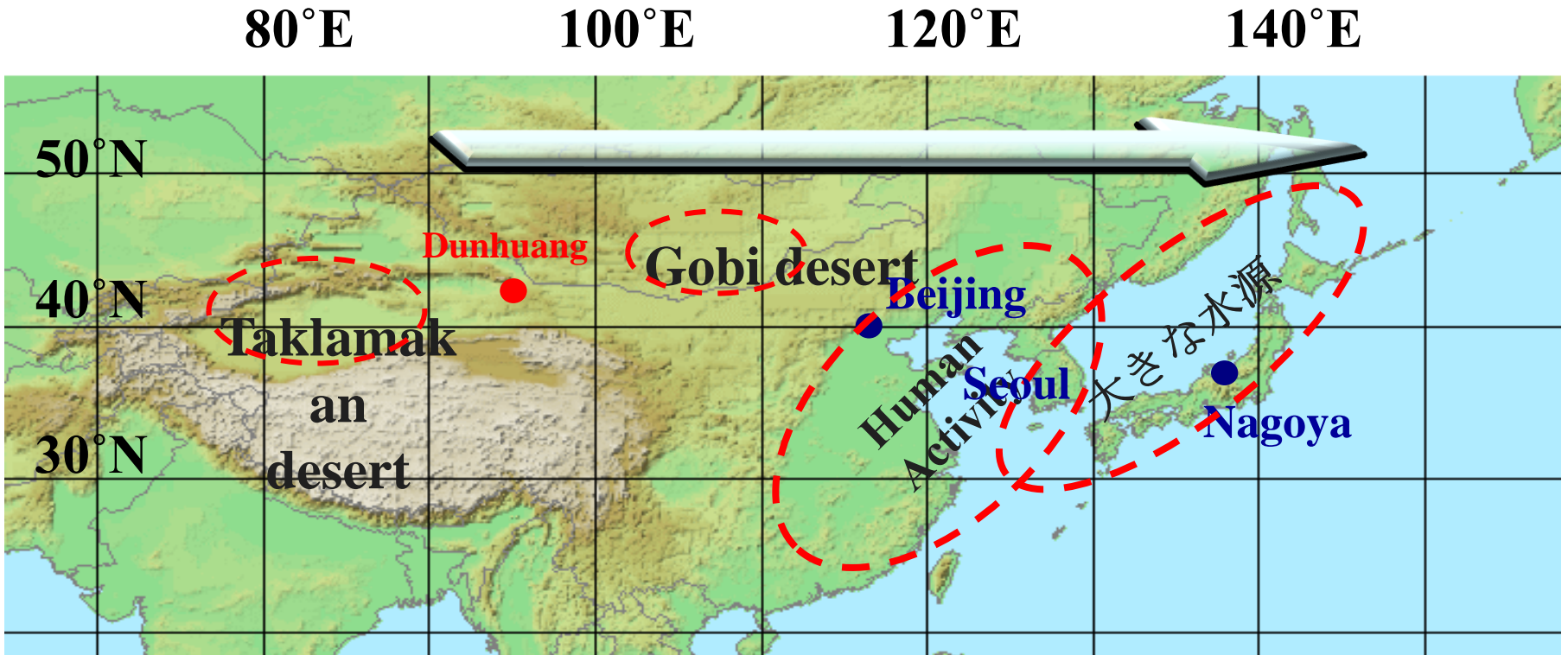
# Asian dust attracts large interest of investigators

2000-2001 (2004) International project of ACE-Asia was made, and many investigators operated their particle collector at many observational sites.



2005年～関係国は観測研究体制を整備、得意分野中心に観測施設の充実





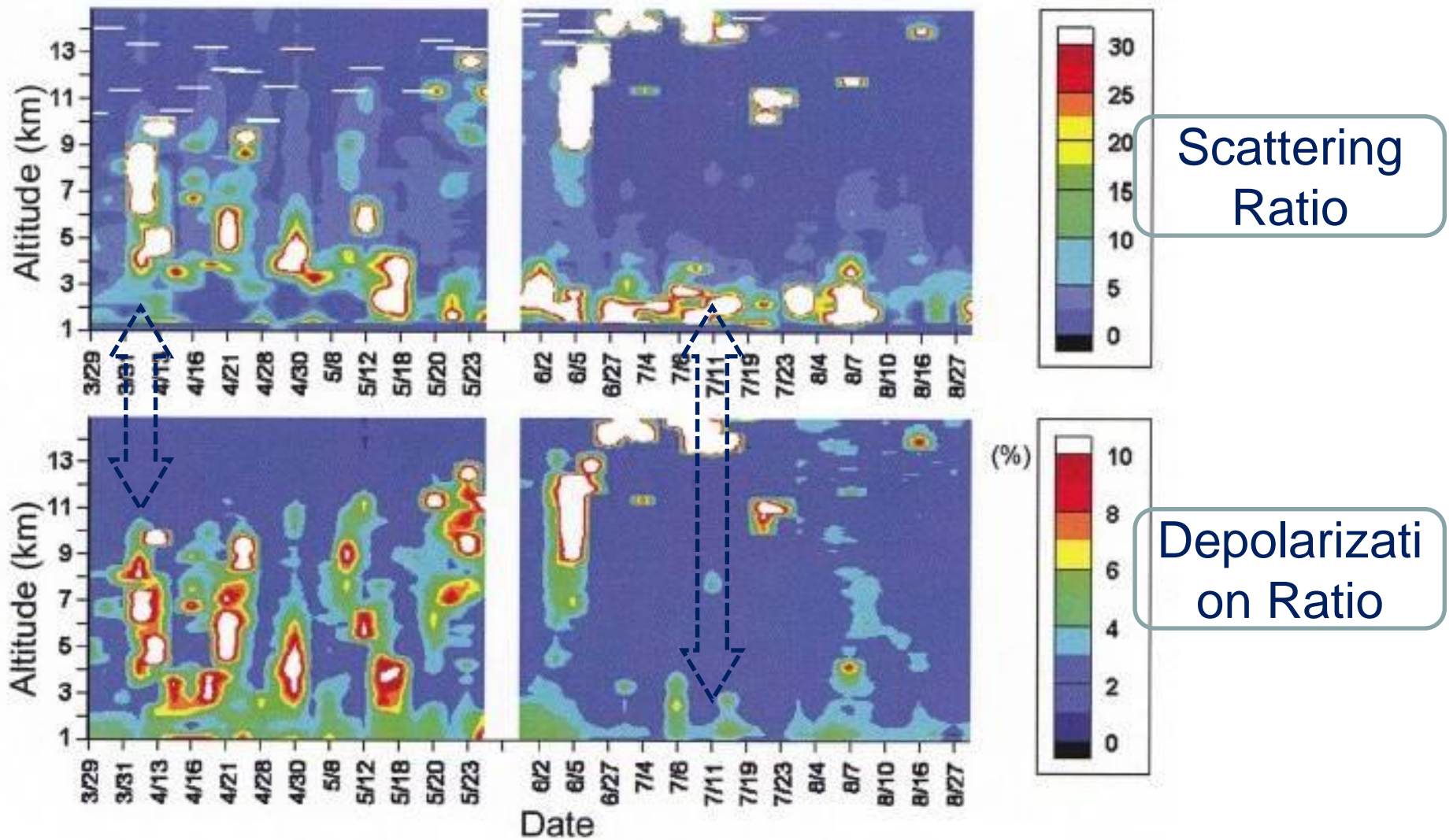
Westerly wind is dominant  
In north-east Asia

Arid region of the Asian continent  
makes lots of atmospheric dust particle

Long-range transport of dust particle  
disturbs the urban atmosphere of big  
cities

# KOSA

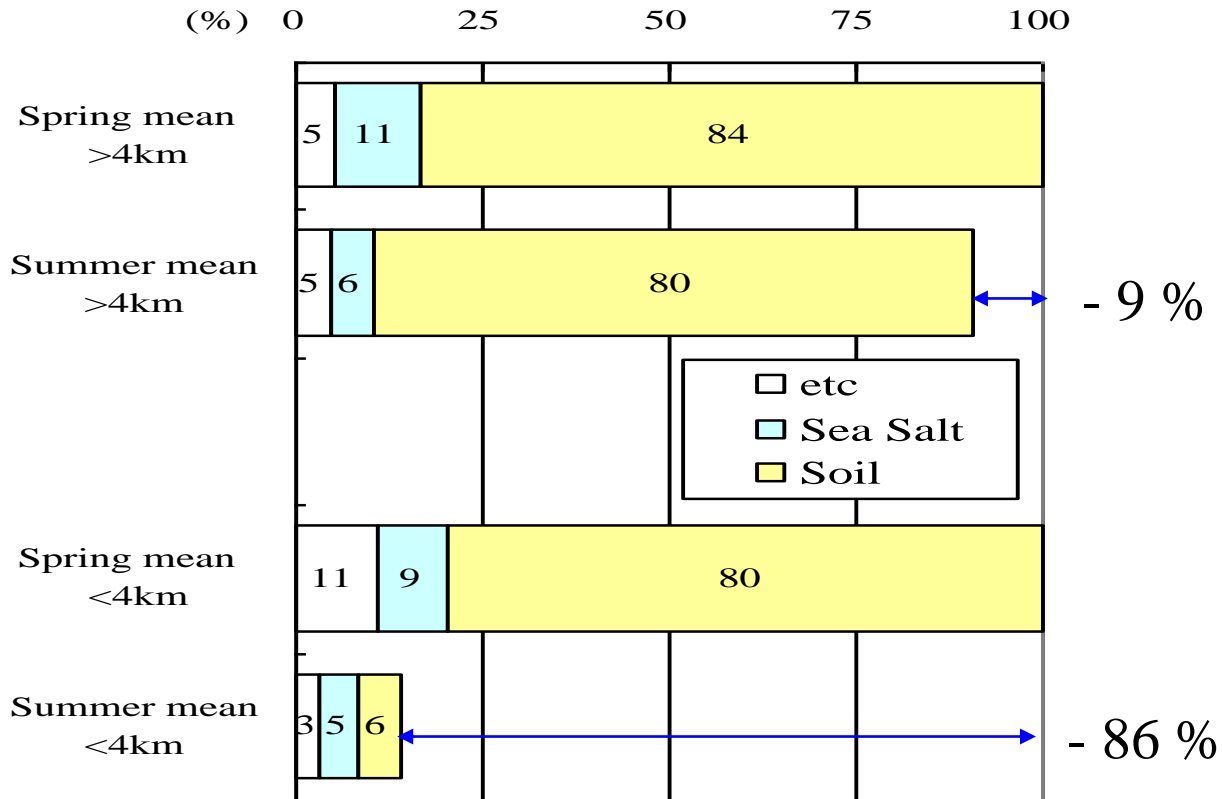




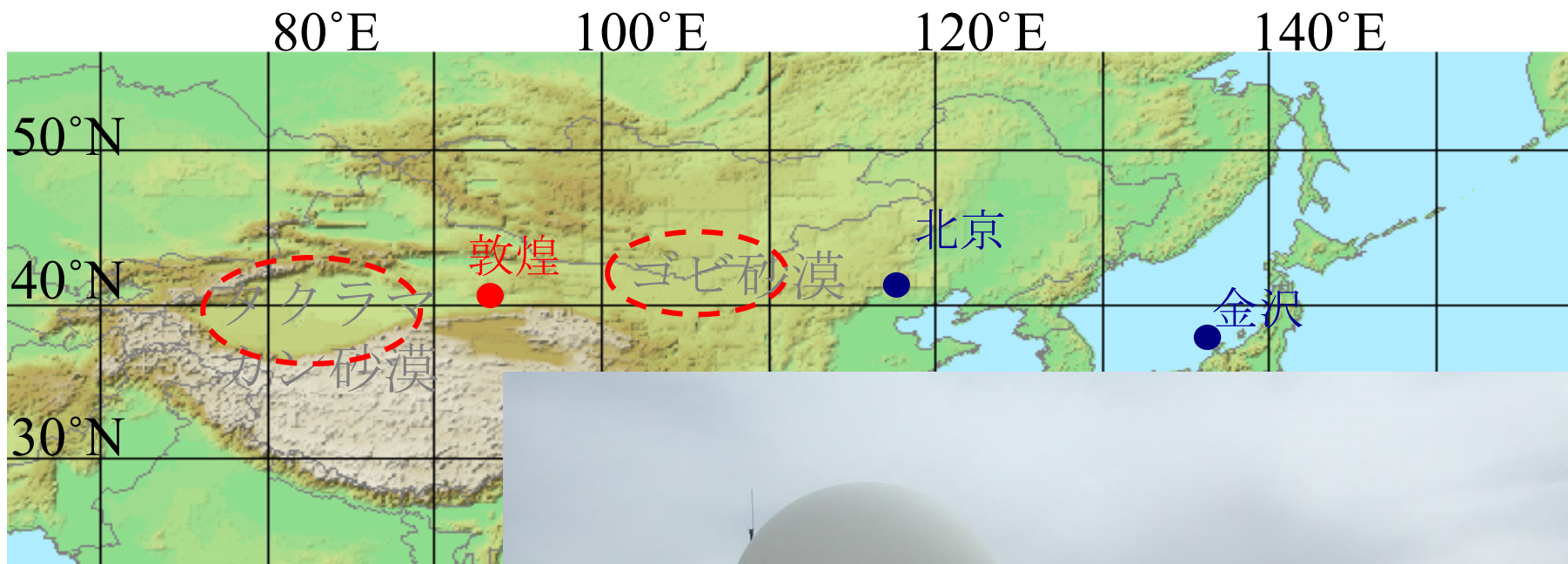
**Figure 9.** Seasonal change in the vertical profiles of the scattering ratio at a laser wavelength of 1064 nm (top panel) and the aerosol depolarization ratio at 532 nm (bottom panel) derived from a series of lidar measurements in Nagoya (35°N, 137°E) during the period of March to August 1994. Tropopause heights are indicated by white horizontal lines in the top panel. A vertical line divides the spring and summer months.

Those measurements were made by Dr. Kwon et al.  
(Kwon et al., Atmos. Environ., 1997, Matsuki et al., JGR, 2003)

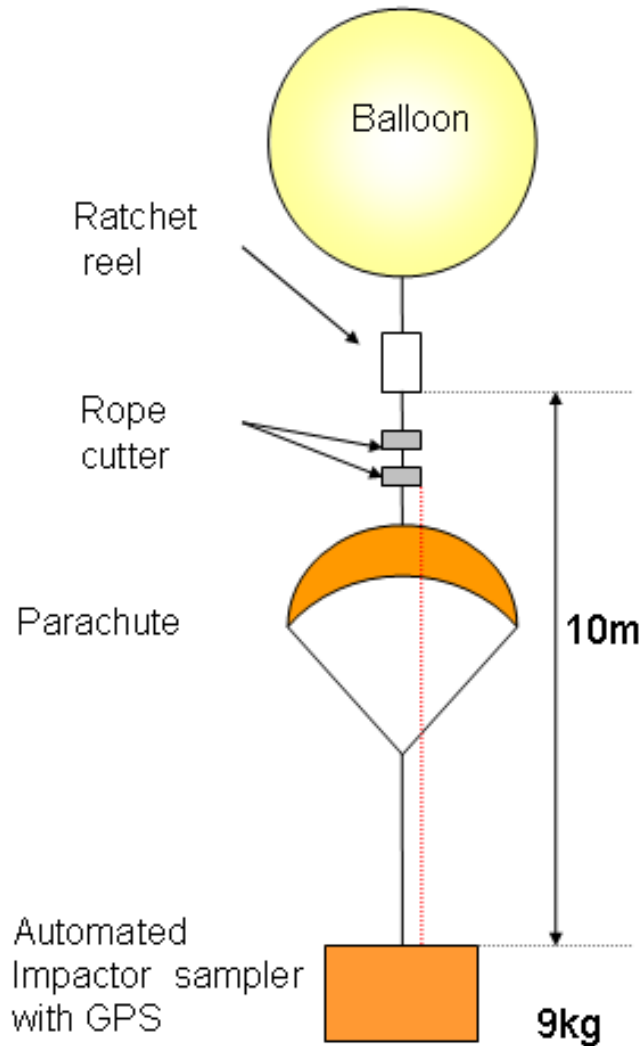
# Balloon and Air-craft borne measurements showed Large effect of westerly wind on global diffusion of KOSA



Observed seasonal change in the vertical structure of coarse ( $D > 1\mu\text{m}$ ) aerosols over Japan. Relative seasonal change in the total number of coarse particles is shown as 100% being the mean value for spring. Fractions by



- **Dust source**
- **Long-range**
- **transportation**
- **Background KOSA**
- **1995 Balloon system**  
was modified for  
downsize
- **1999 Dust operation**  
started



Let's consider  
advantage of balloon  
measurements

Balloon-train

Positioning of sampler  
is made  
by GPS

**For single particle analysis, particle collector system should be recovered!!**



Westerly becomes  
clear  
above about 5km

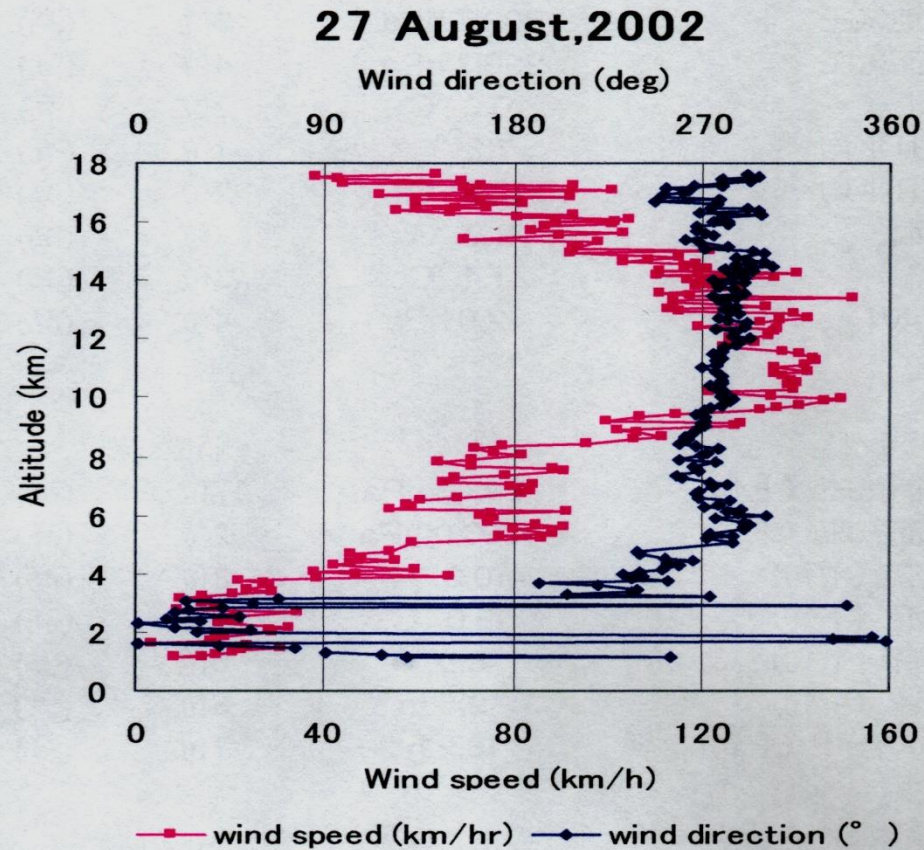
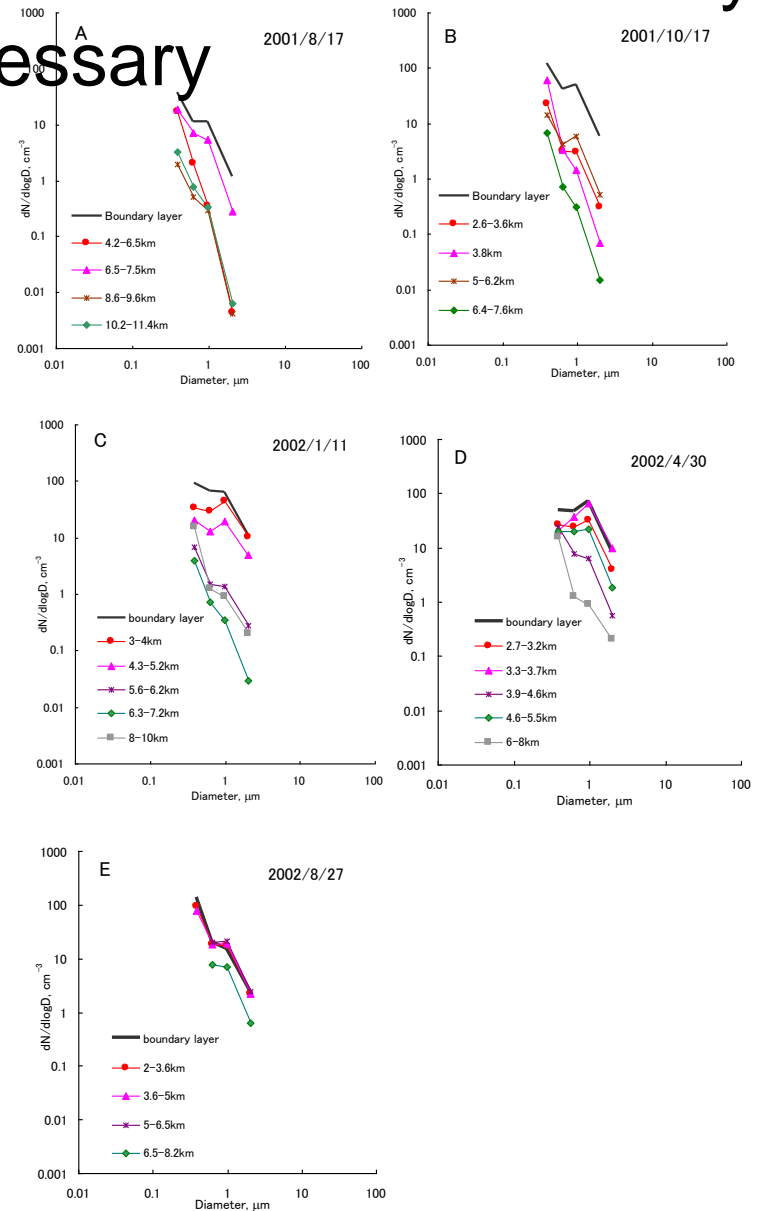
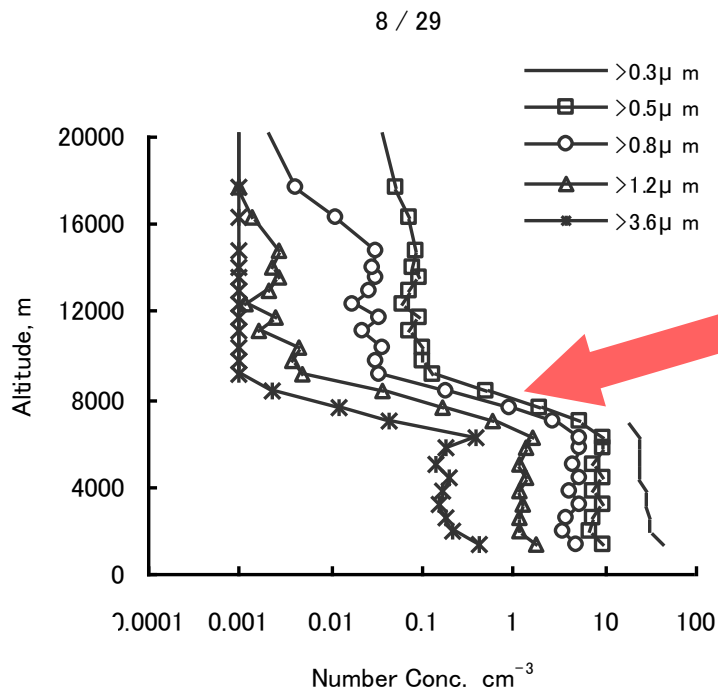


Fig.6 Wind speed and direction deduced from analysis of the balloon trajectory.



# Particle concentration is measured by Balloon-borne OPC and balloon-recover is not always necessary

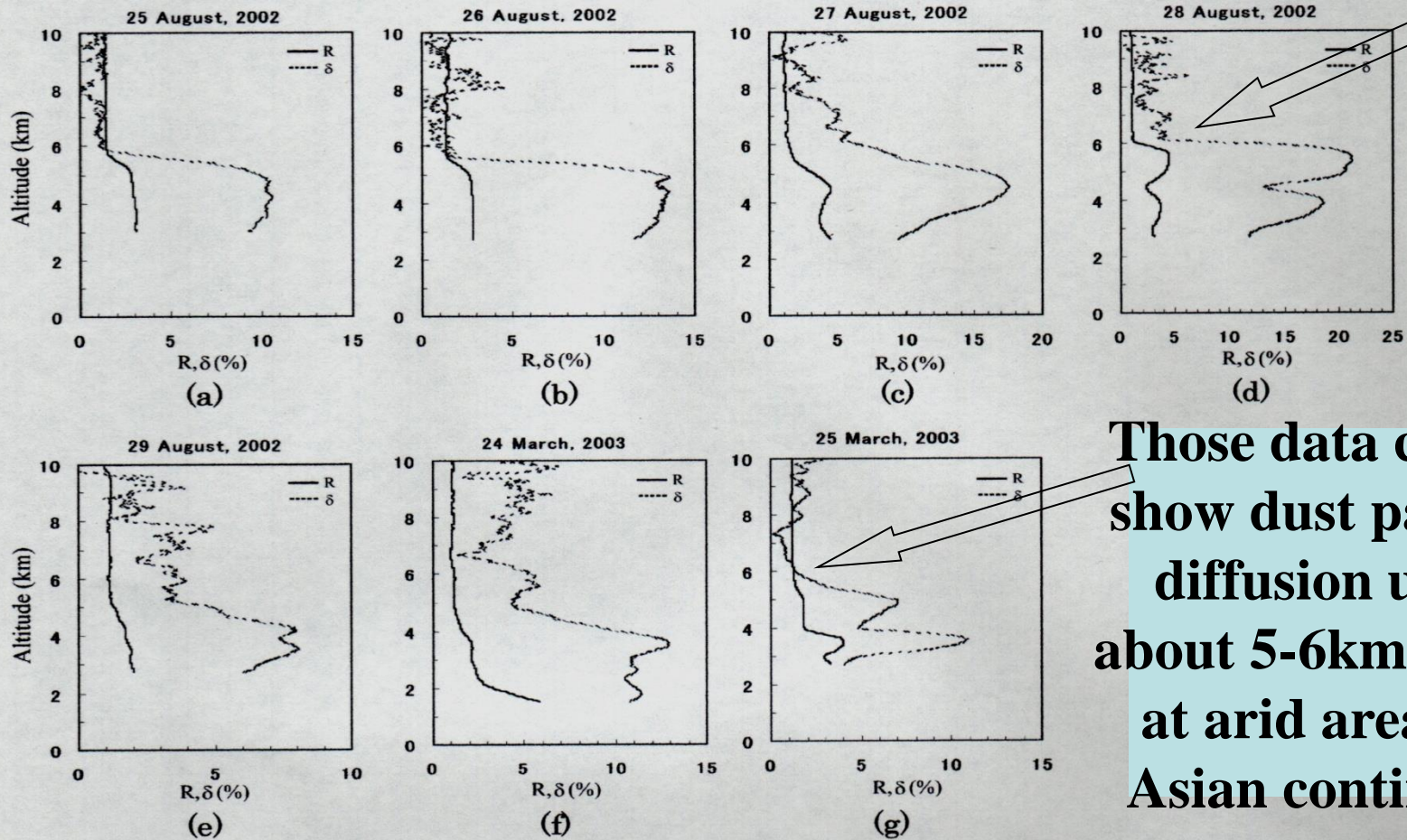
necessary



気球による直接観測で5~6kmに  
ギャップがしばしば見つかる

1/19/2016

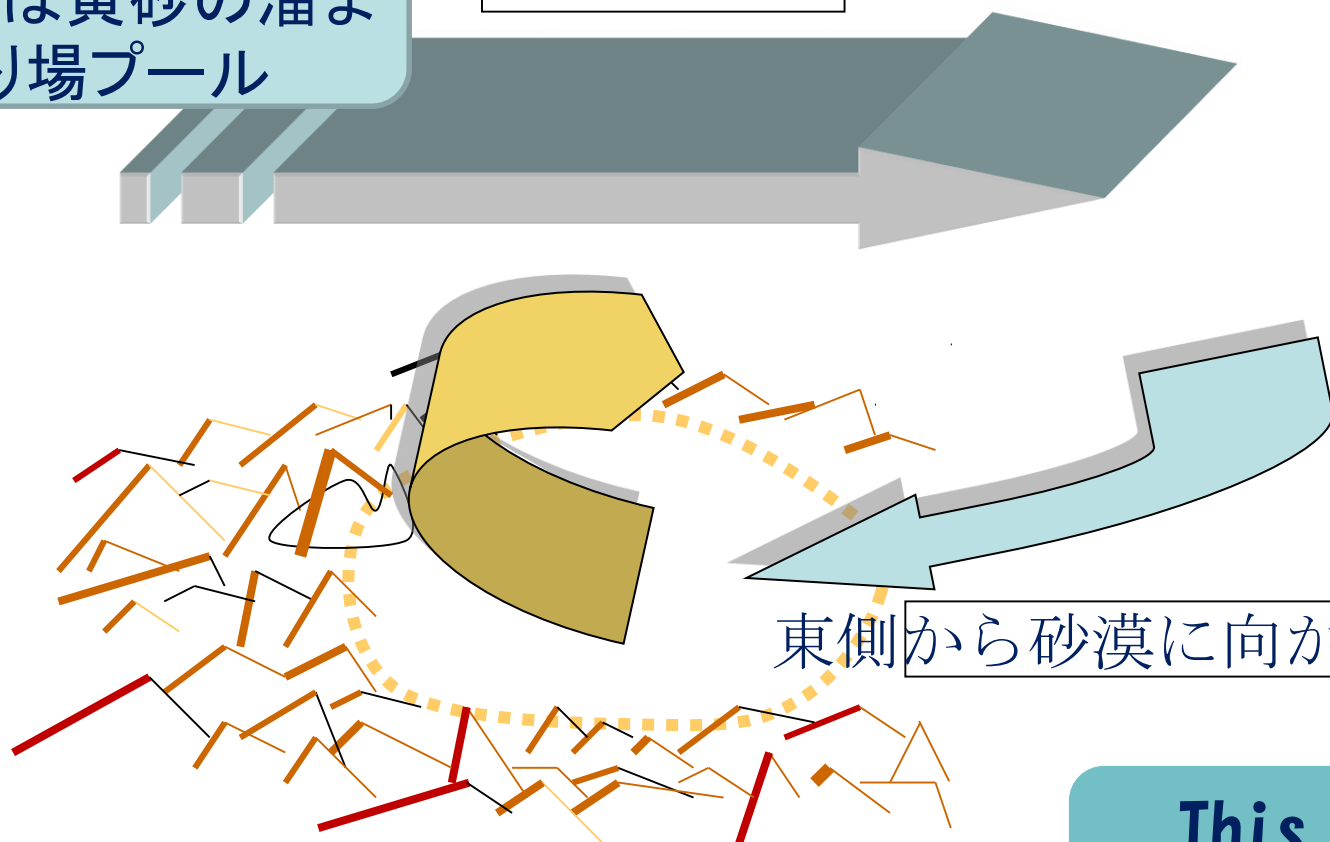
# Vertical Profiles of Aerosols Measured by Lidar at Dunhuang (Iwasaka et al., JGR, 2003)



Those data clearly show dust particle diffusion up to about 5-6km height at arid areas of Asian continent

タクラマカン砂漠  
上空は黄砂の溜まり  
場プール

上空の偏西風

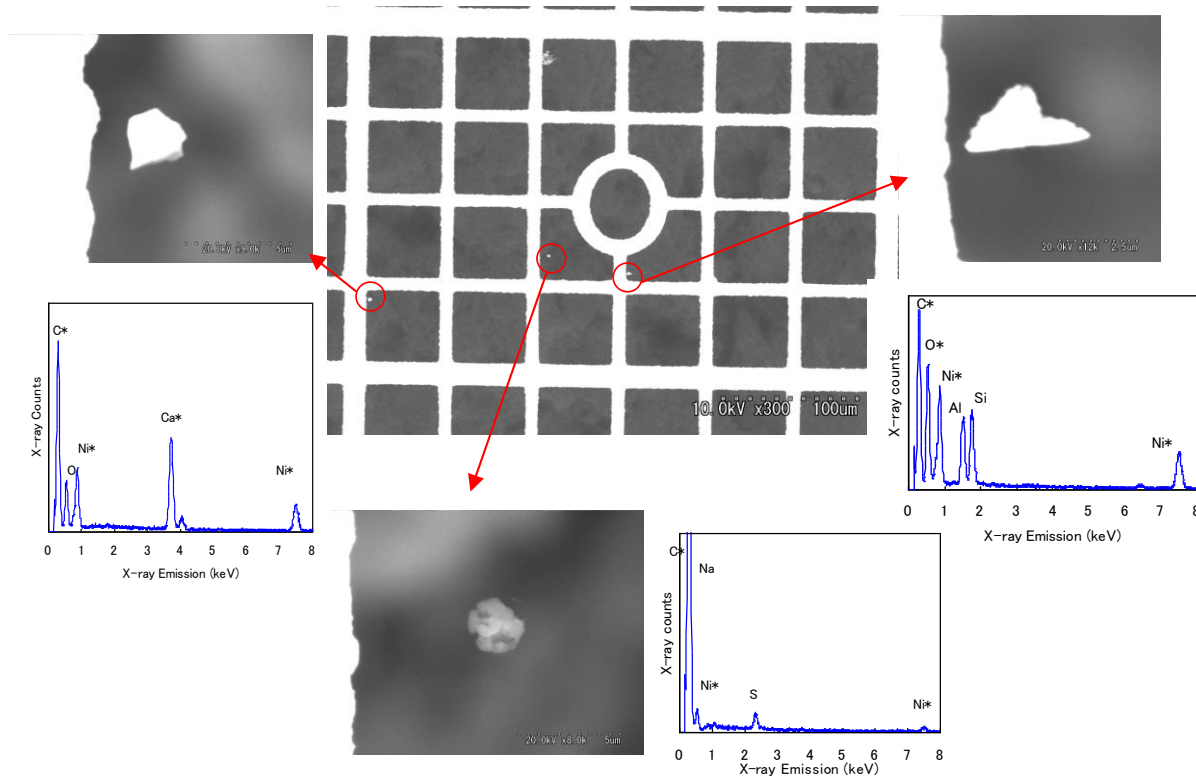


東側から砂漠に向かって吹き込む

This causes  
Background KOSA

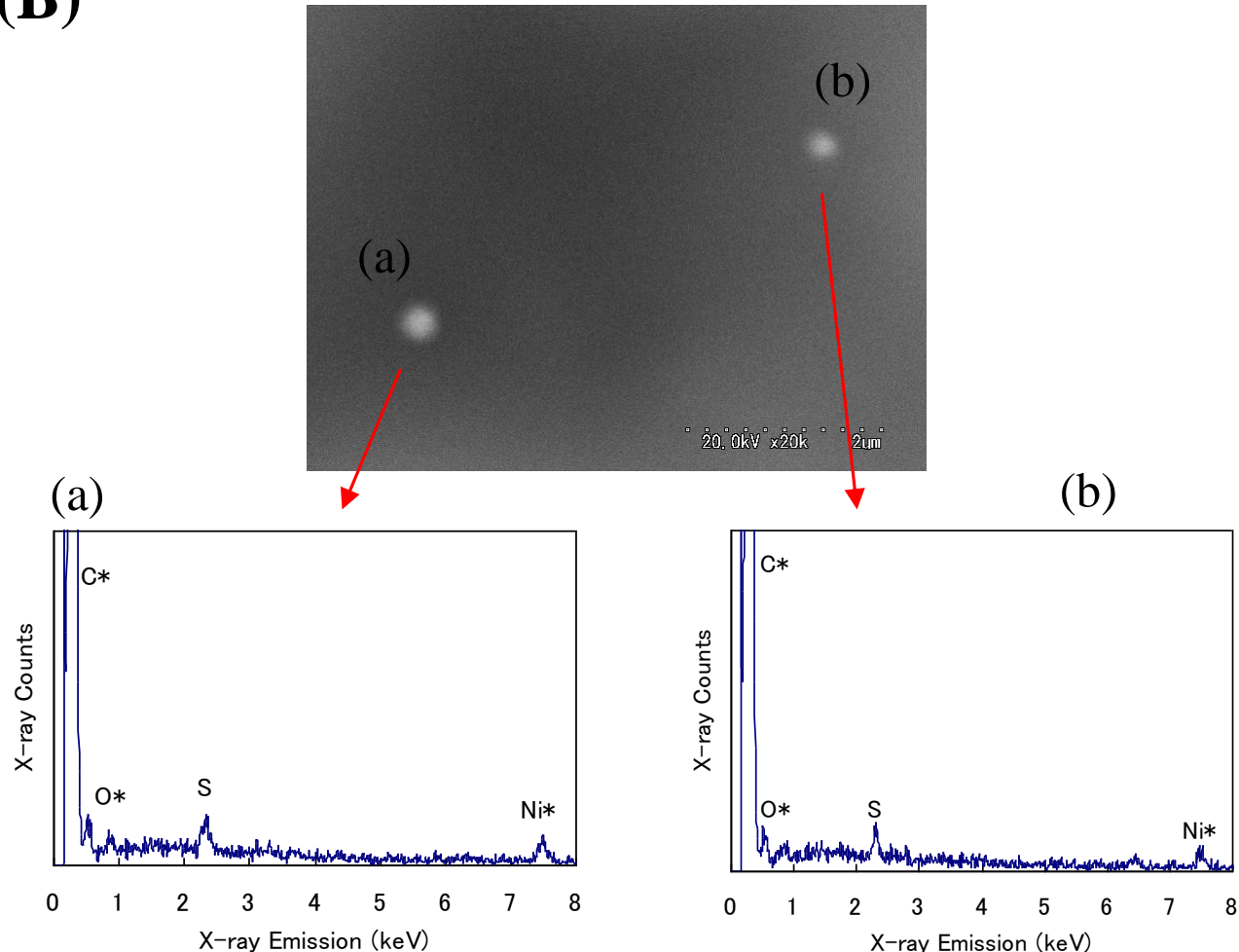
タクラマカン砂漠では  
平均高度4000mの山々に囲まれて地上には出口が  
東側にしかない。山谷風で巻き上った砂塵は、上  
空へ拡散する。ここから偏西風で風下へ拡散する。

# Coarse Particles in the Free Atmosphere, 3-5km at Dunhuang in summer of 2002



# Fine particles also were collected

(B)

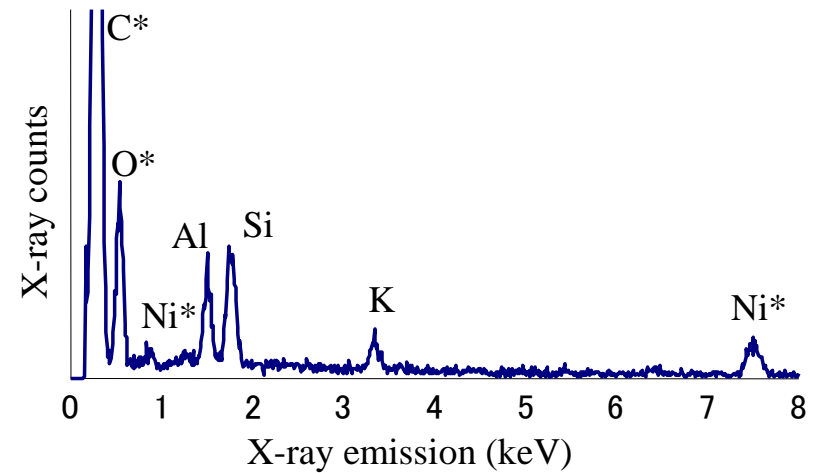
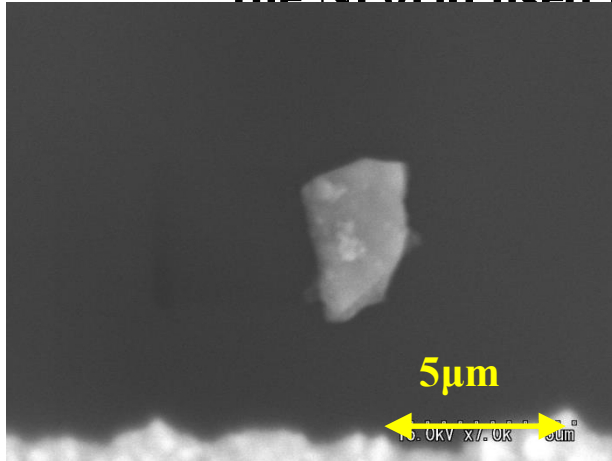


**Electron micrograph of individual particles collected in the free troposphere between about 3km and 5km over Dunhuang, China. Both fine particles (a) and (b) are ammonium sulfate.**

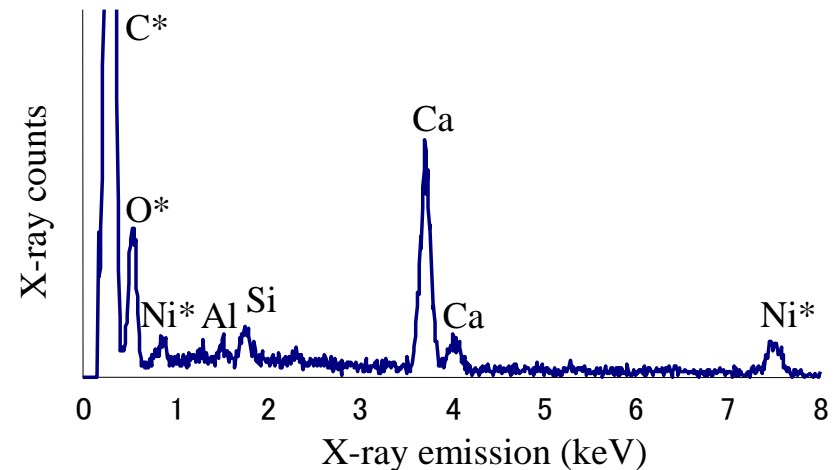
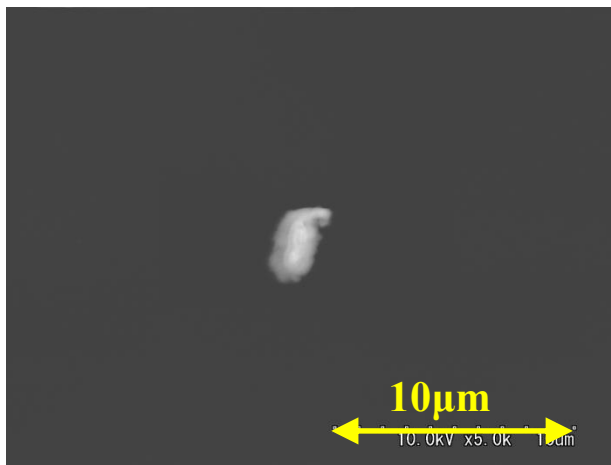
# Typical electron micrograph of collected particles in spring in the range of 3-7km.

(a) Si-rich particle, (b) Ca-rich particle. The Ni\* peak is due to the Ni grid used inside the collection surface

(a)



(b)



# バイオエアロゾルの対流圏広域拡散による影響

微生物生態系への影響、動植物に対する病原性

(e.g., Griffin et al., 2001; Prospero, 2005; Kellogg and Griffin, 2007)

氷晶核として雲形成や間接放射に影響

(e.g., Maki and Wiloughby, 1978; IPCC, 2001; Möhler et al., 2007)

氷晶核としての能力

バイオエアロゾル

-7 ~ -1 °C

(Möhler et al., 2007)

-2 ~ -4 °C

(Shenell and Vali, 1972)

鉱物粒子

-13 ~ -20 °C

(Isono et al., 1959)

スノーマックス

(凍結剤)!

# IPCC 2001 report suggested the possible contribution of micro biota to climate/environment disturbance

## Example 5: Pollen

**Size:** around 3 to 100  $\mu\text{m}$

**Sources:** plants and vegetal material

**Appearance:** many different shapes.

**Lifetime:** hours to days. Pollen are effectively removed by precipitation

### Other properties :

- little water soluble
- health problem for persons suffering from allergies (hay fever).
- Ice nuclei ?

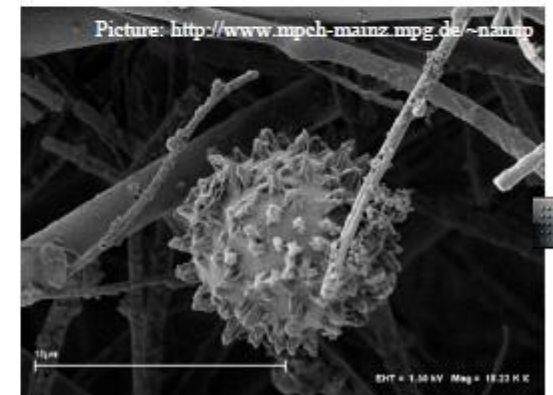


Fig. 1.28 Pollen



# 中国敦煌市にも観測サイトを経営

黄砂発生源地、黄砂沈降地域の系統的な比較を目指す



2006年サンプリング

2007年サンプリング

Bioaerosols seem to have large potential affecting global environment.

Water cycle  
Biogeochemical cycles

Knowledge desired now

1. Vertical changes in Mixing ratio of bioaerosols to total particles
2. Size dependence of bioaerosols (and Mixture)
3. Behavior of those particles in the free troposphere is important

# SEM-EDX (対象 Dp>1um)

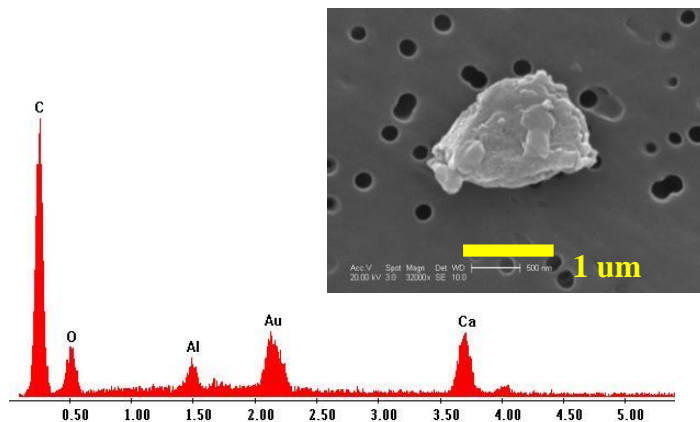
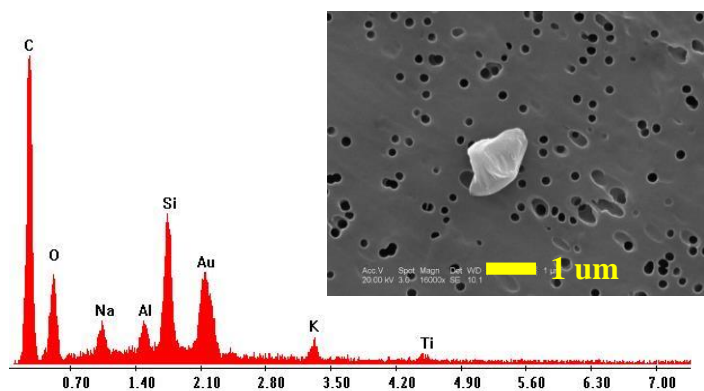
分類

鉱物粒子: 検出元素により同定 (Si, Ca, Fe, Na, MgまたはTiを含有)

微生物: 検出元素 (鉱物粒子以外でC-rich、またはリン含有) と形態により同定。

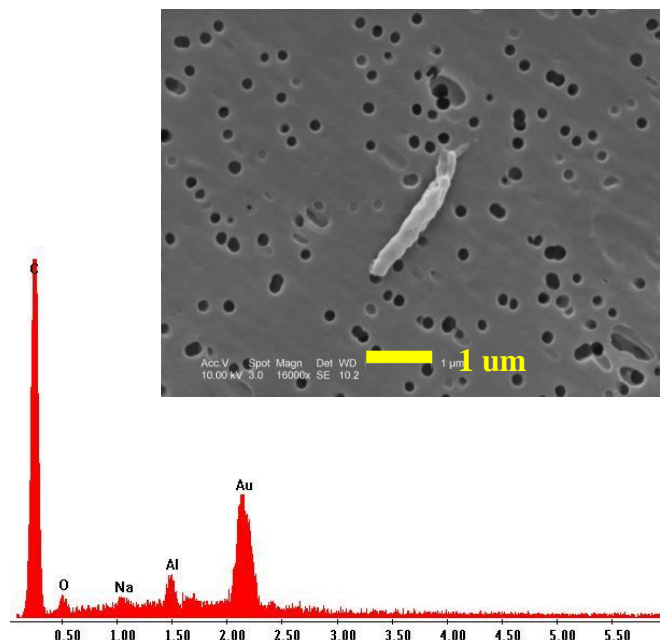
その他 (バイオマス燃焼由来、人為起源重金属)

## Mineral dust particle



~ 99% (149/150)

## Bacteria-like particle



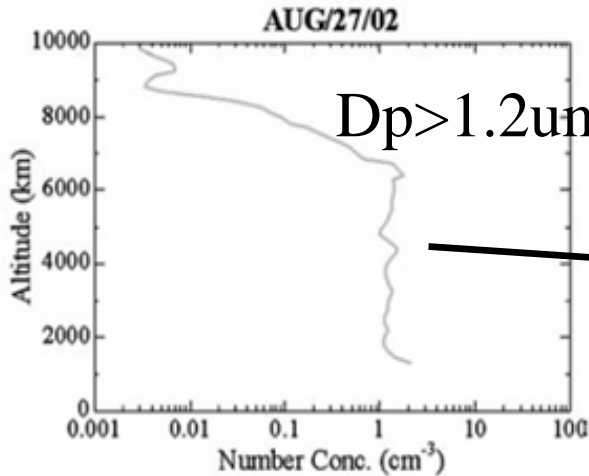
Only one particle (1/150)

分析数(>1um): N = 150

# 微生物が付着している鉍物粒子の個数濃度 ( $d > 1 \mu\text{m}$ , 700m, Aug.17, 2007)

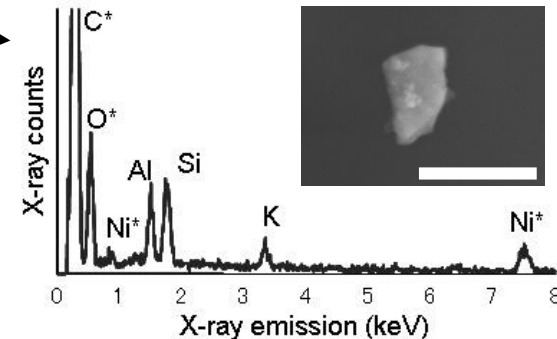
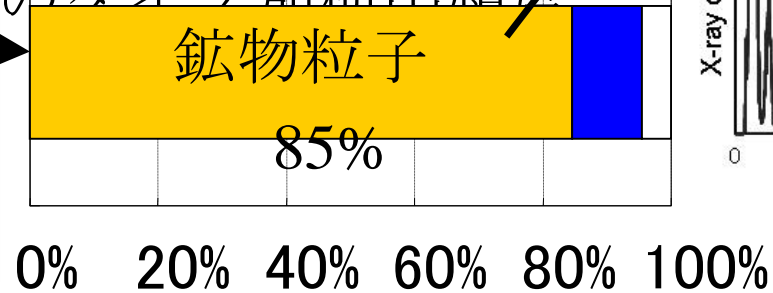
粒子数濃度 ( $d > 1 \mu\text{m}$ )	鉍物粒子の検出頻度	微生物と内部混合している比率	微生物が付着している鉍物粒子の個数濃度 ( $d > 1 \mu\text{m}$ )
$2.5 \times 10^3 / \text{L}$	100 %	10 %	<b><math>2.5 \times 10^2 / \text{L}</math></b>

## 粒子数濃度の鉛直分布



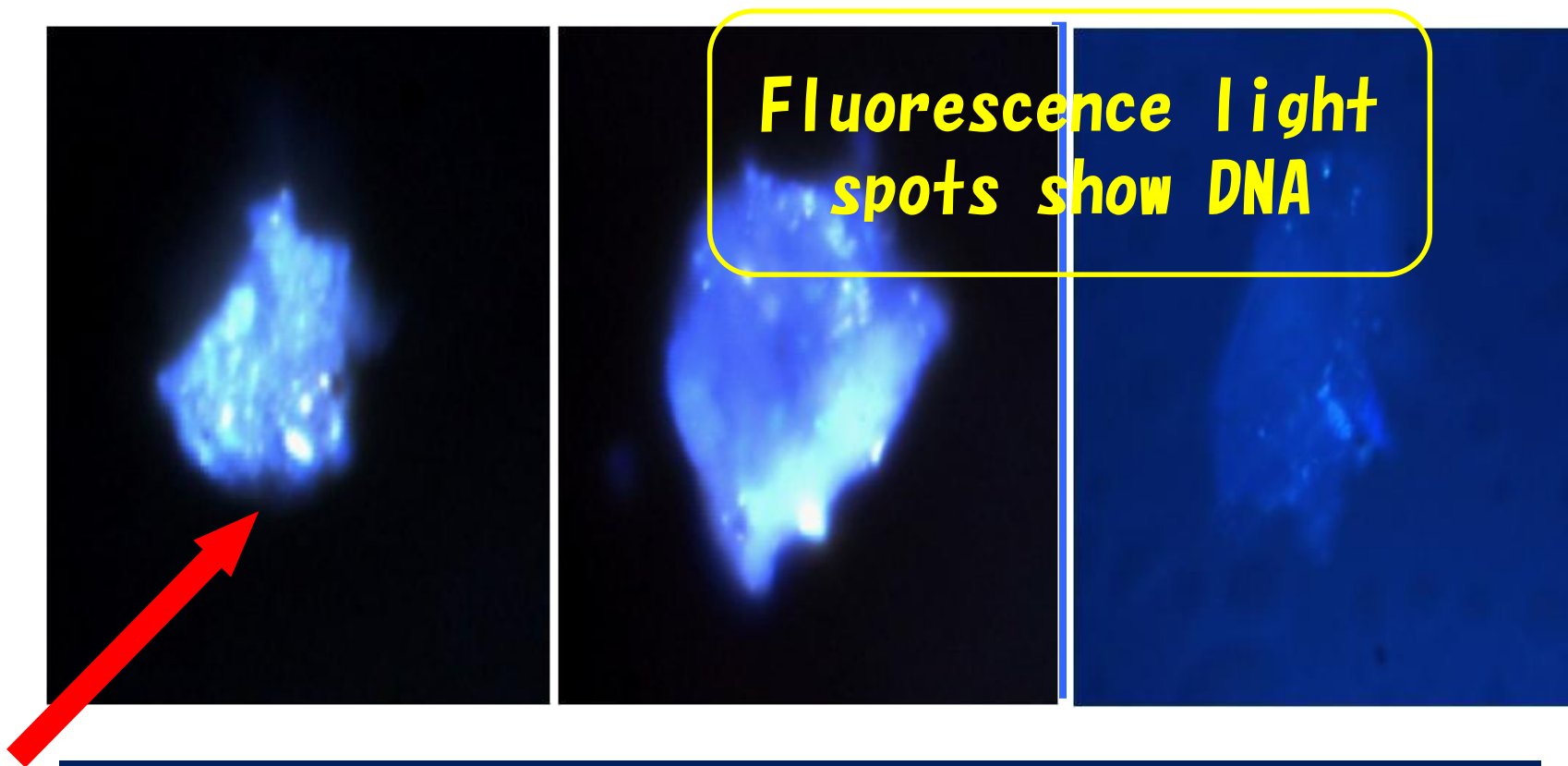
敦煌上空のエアロゾルの

タイプ別検出頻度



鉍物粒子は、高度6km辺り（自由対流圏高度）まで定常的に存在

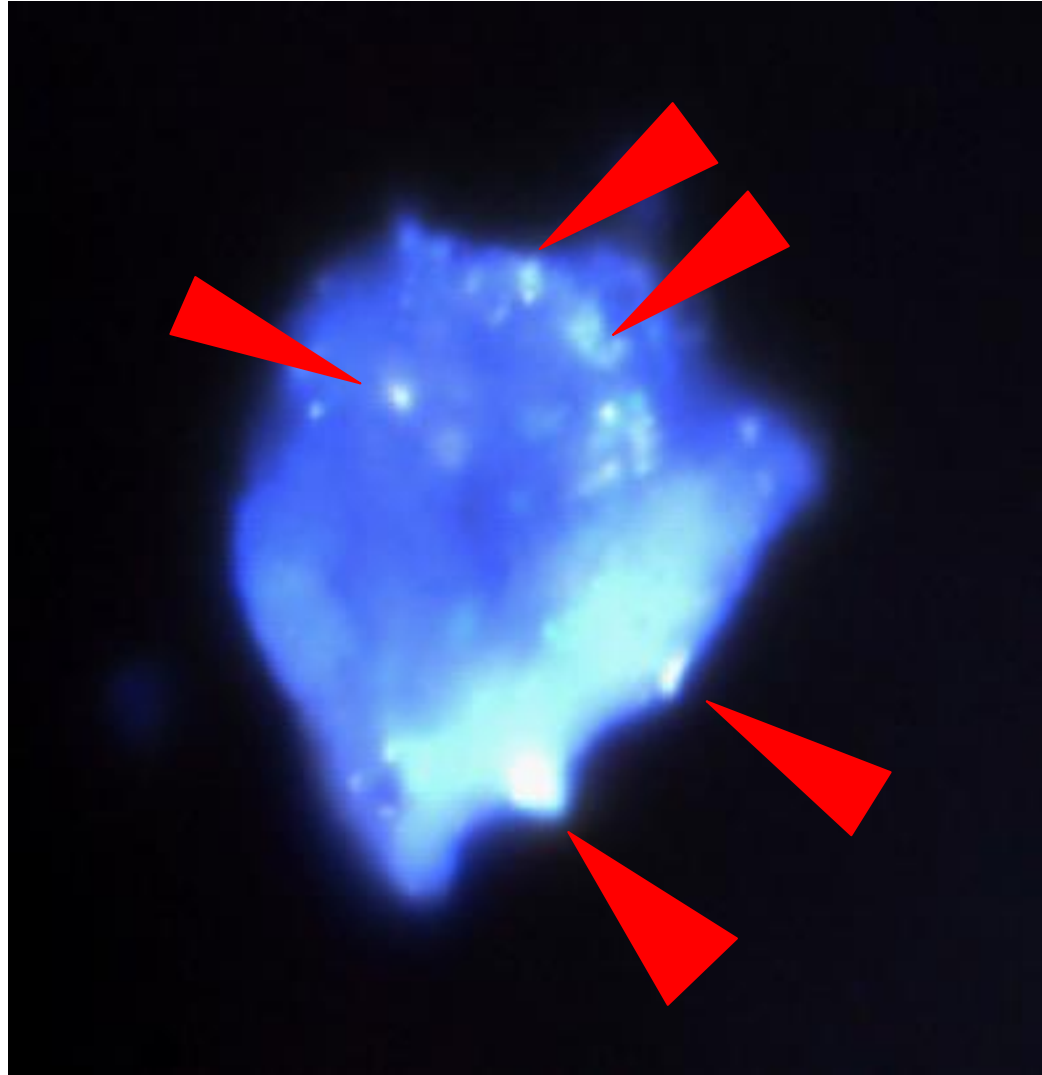
# KOSA-Bioaerosol collected at Dunhuang: DAPI Treatment

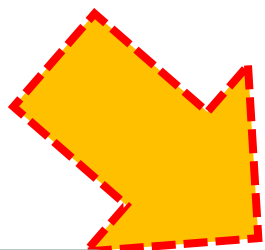
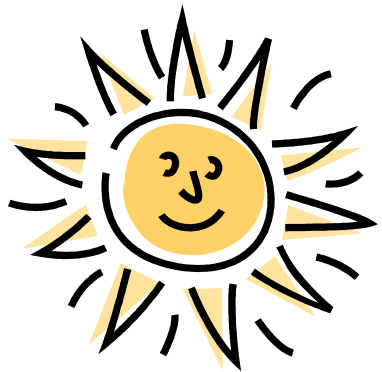


Strong Fluorescence Light is observed on Dsut Particle Surface

2007年敦煌 (地上700 m : 海拔1900 m)

Maki et al., 2008,





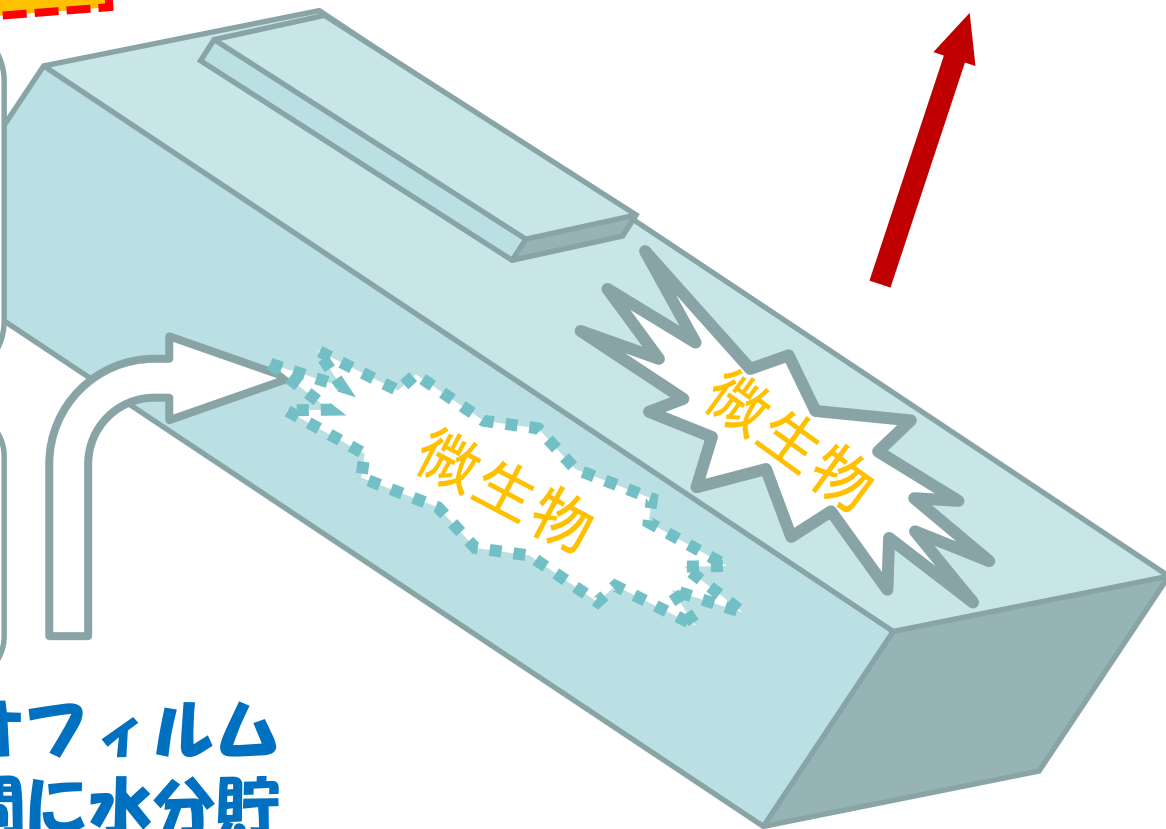
太陽紫外線  
ストレスを  
低減

黄砂の氷晶核能力を  
高める！

黄砂を住み家  
に長距離輸送

微生物が受け  
る環境ストレス

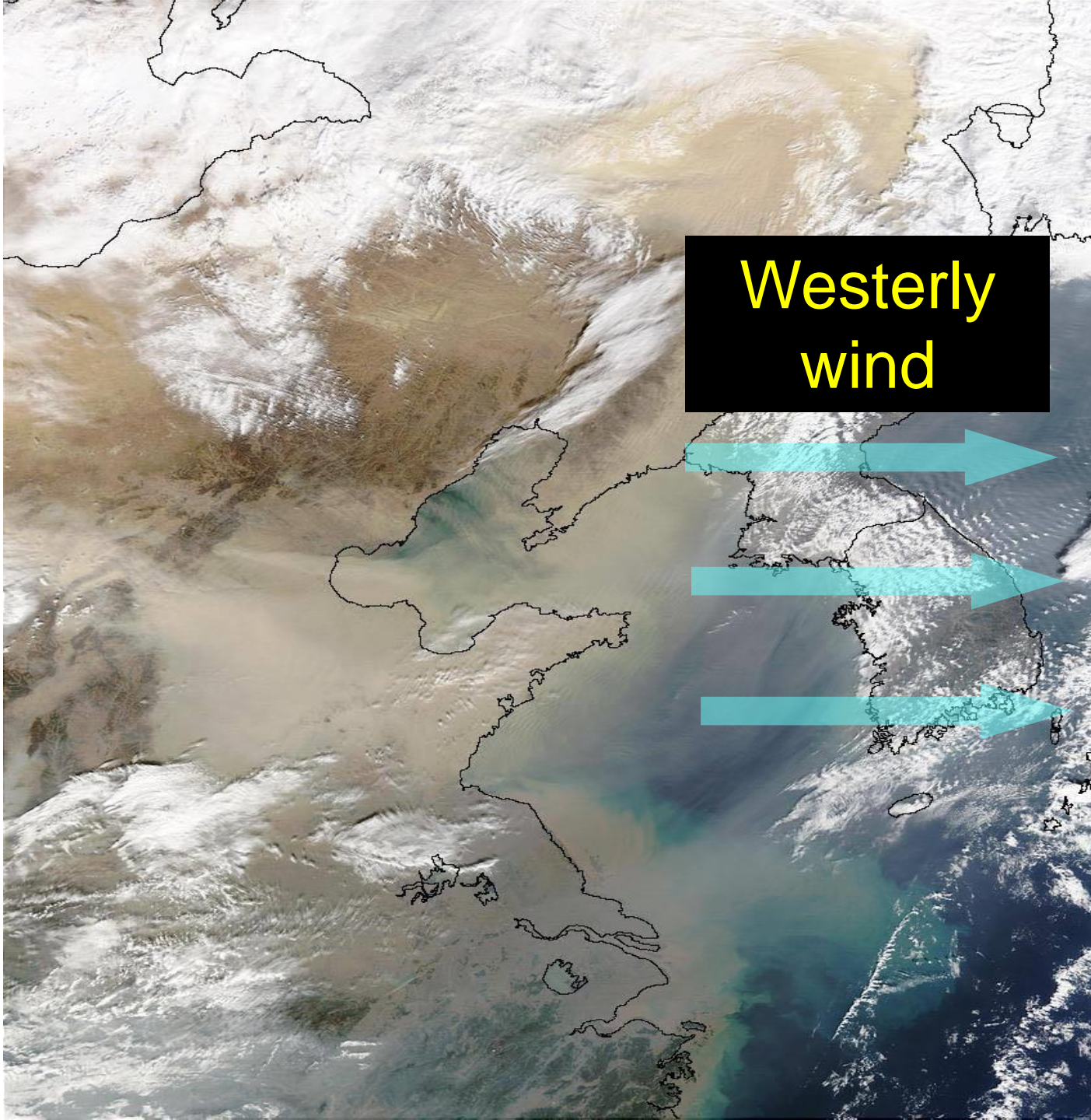
バイオフィルム  
との間に水分貯  
めこみ



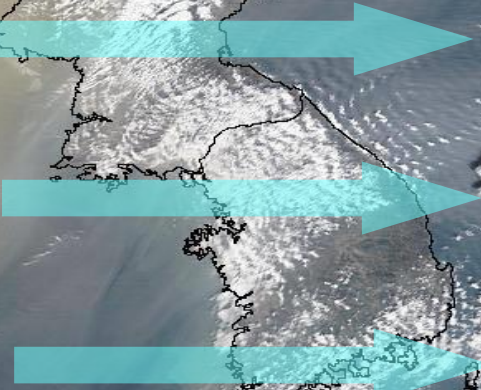
Most recently various kind of bioaerosols (new type aerosols) were identified on the basis of modern biological technique:

Some investigation suggested that micro-organisms are played as effective ice nuclei and/or condensation nuclei. However, those investigations are strongly suggestive, and there are lots of scientific problems. Addition to those, existence of such kind of biology possibly controls nature of atmosphere and we possibly have to make new type concept and/or definition.



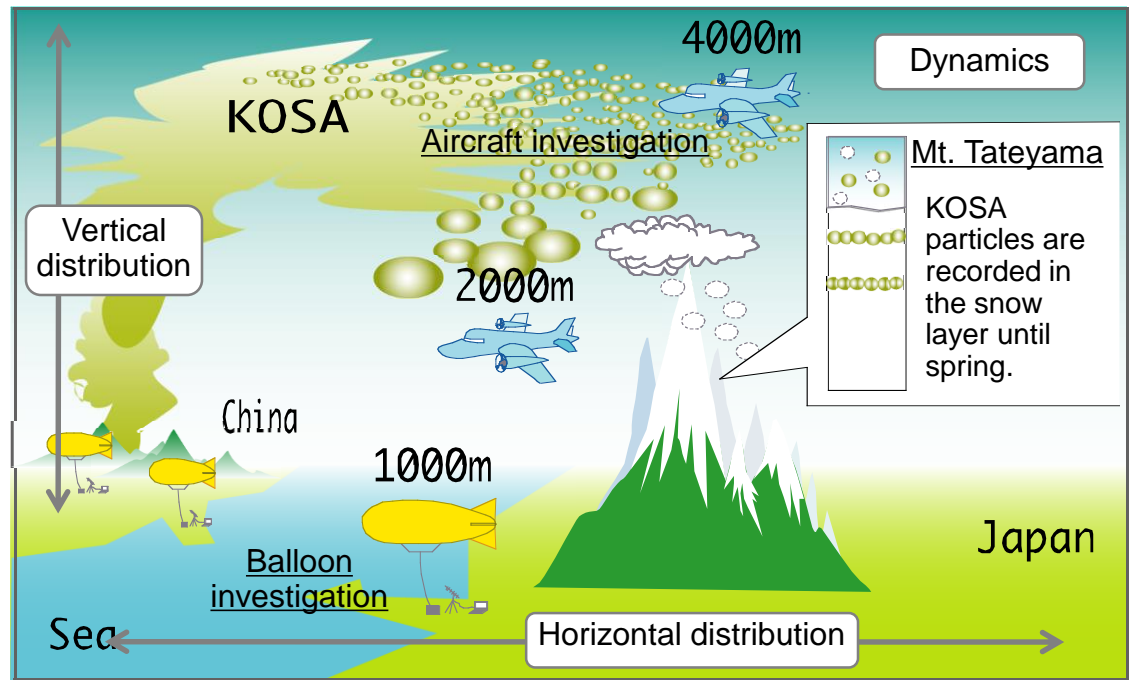
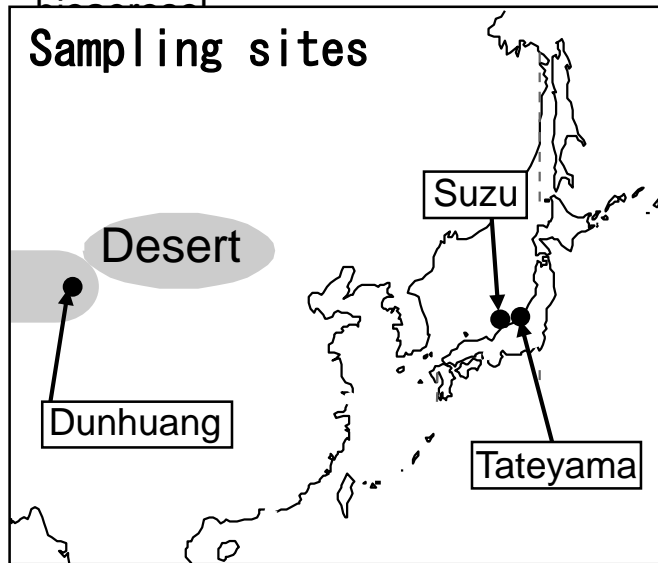


Westerly  
wind



# How to collect aerosol at high altitude.

Comparison of bacterial species among sampling sites for elucidating the long-range transport of KOSA



- Isolation of atmospheric microorganisms.
- Analysis of microbial species composition.
- Understand the microbial characteristics.

# Air sampling in Kanazawa (2011 May)

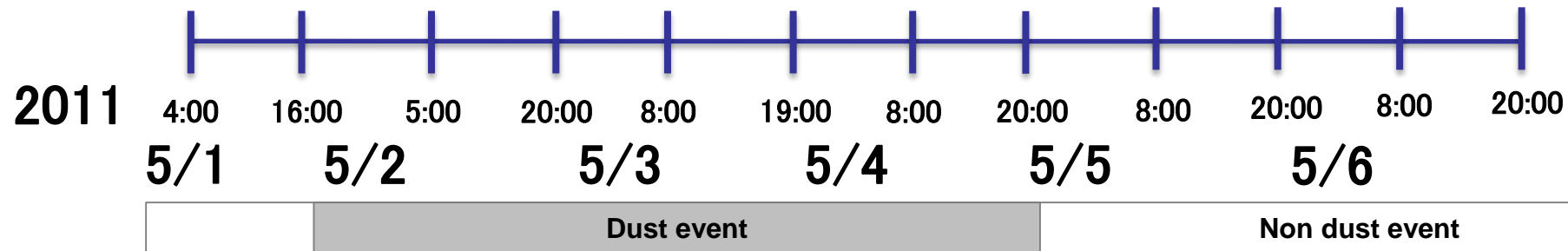
Date : 2011 May 1 - May 7

Site : Roof of Kanazawa Univ.

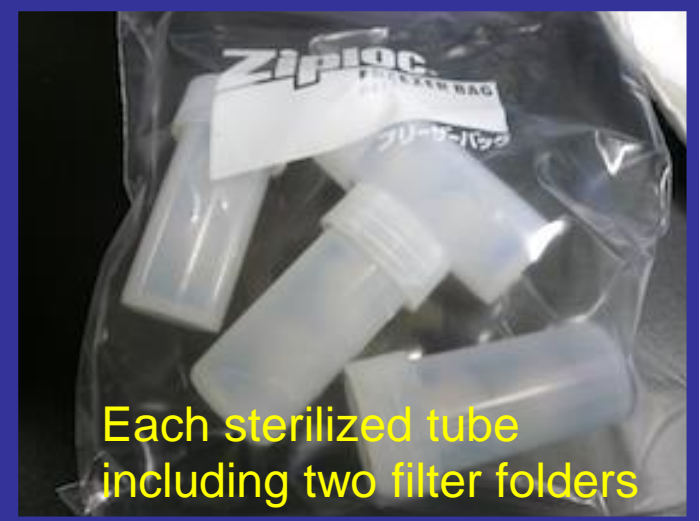
Altitude : 10 m

Method : Collection on 0.2 $\mu$ m  
pore size poly  
-carbonate filter

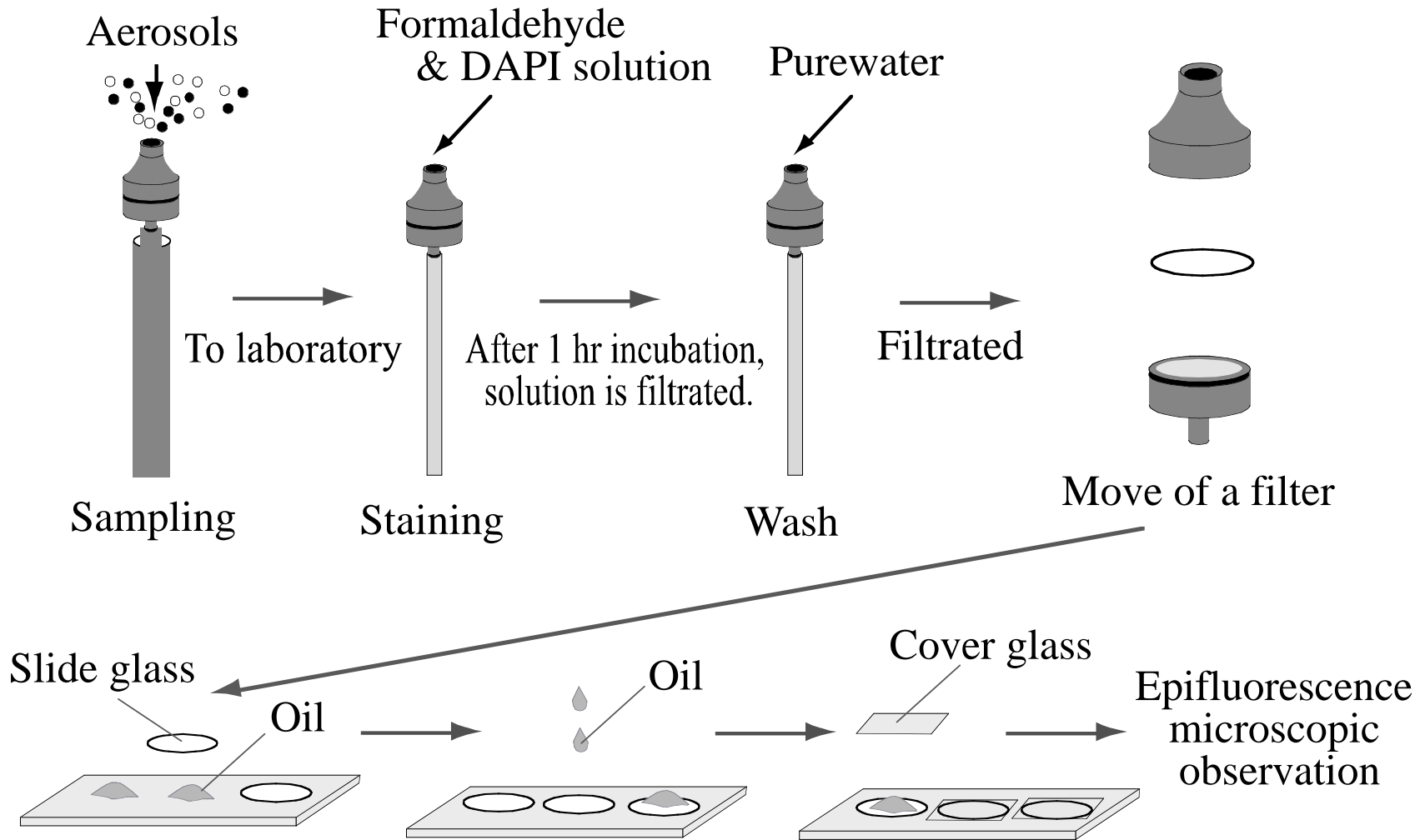
Rate : 700 L/h



# Bioaerosol sampling on buildings (5m~10m)



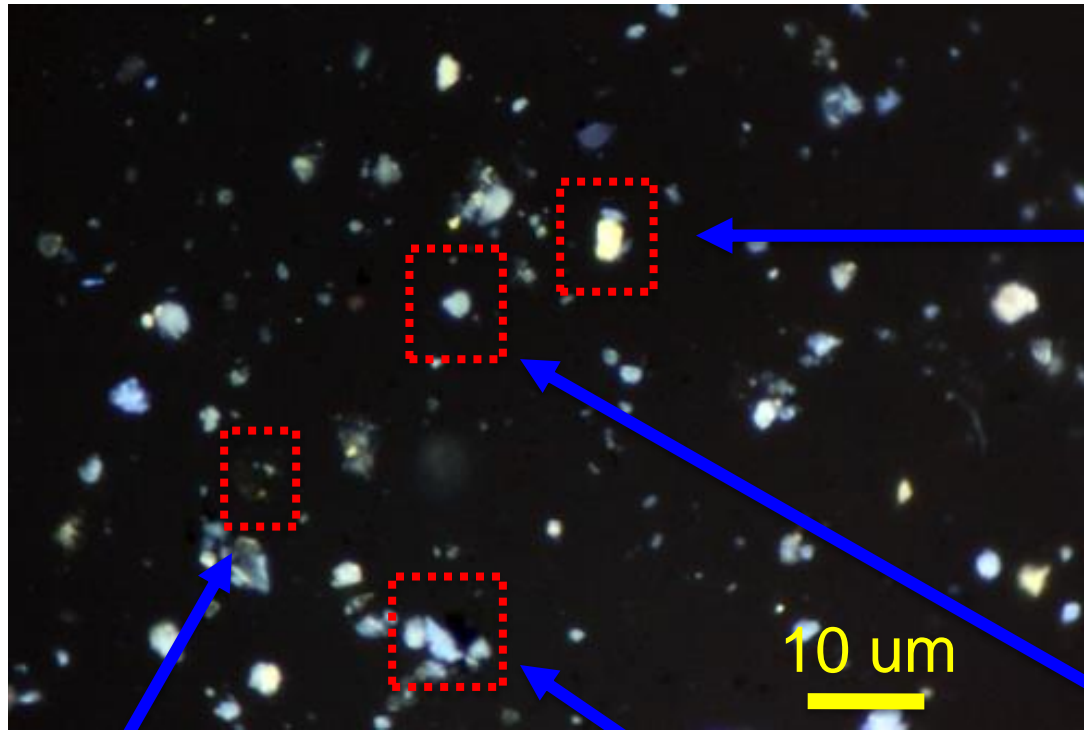
# Sampling method & Approach



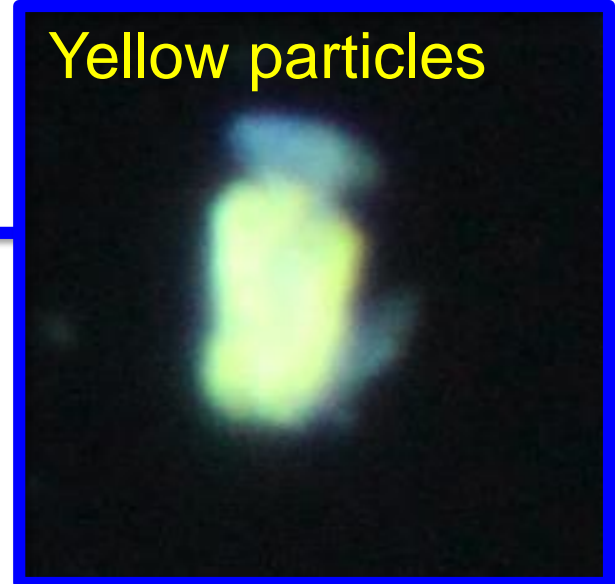
**Microbial abundances** are determined using microscopic observation with fluorescence dye staining.

# Microscopic observation

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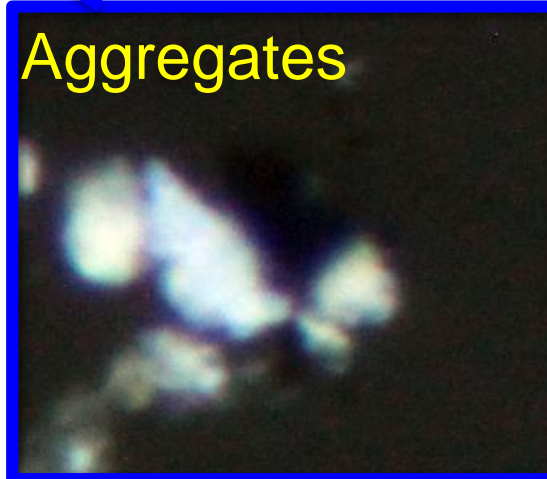
Yellow particles



Bacteria



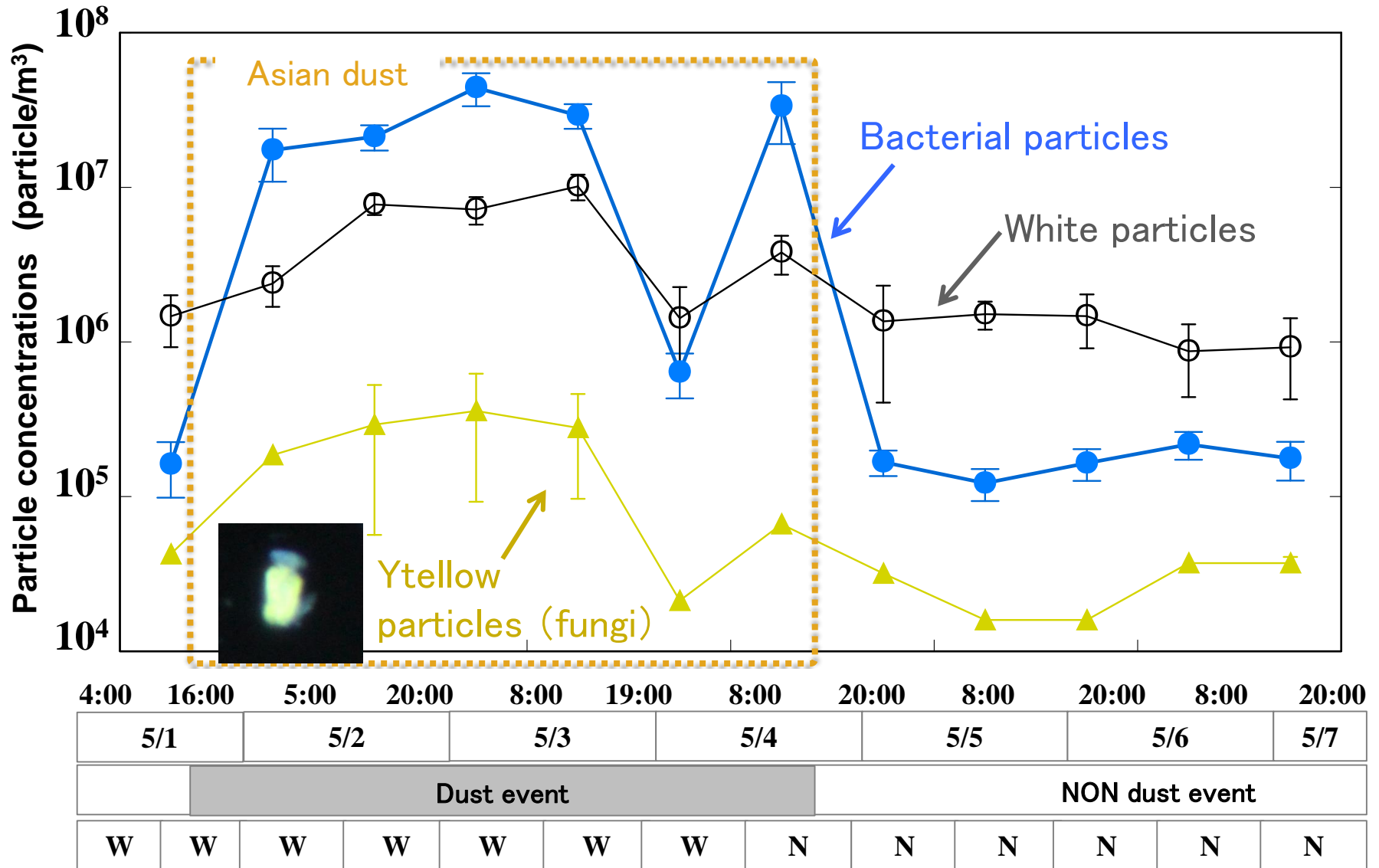
Aggregates



White particles

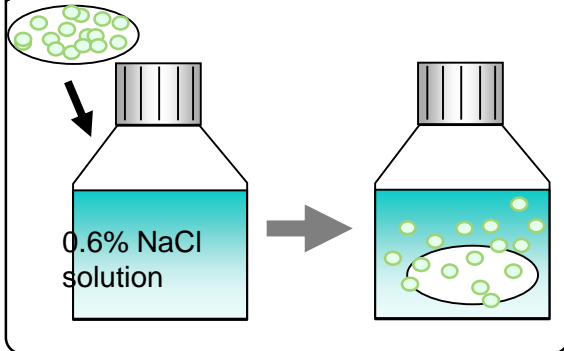


# Changes in particle in concentrations



# 16S rDNA clone libraries

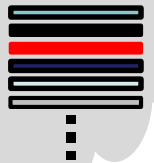
Bioaerosol on filter were washed by liquid solution of 0.6% NaCl solution.



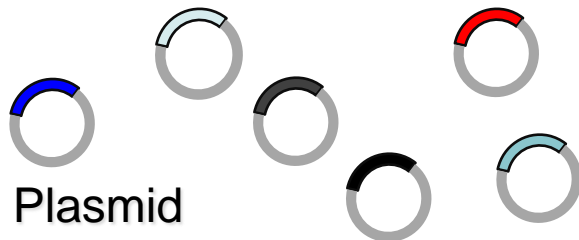
Extraction of genomic DNA

PCR targeting 16S rRNA gene

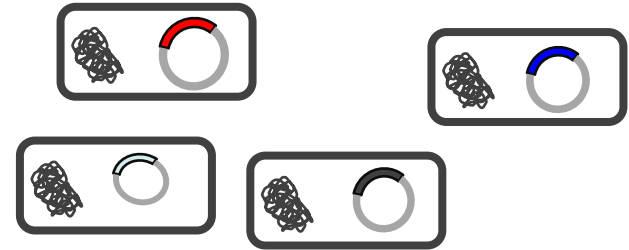
Heterogeneous rRNA gene.



Cloning : each rDNA product are transformed to *E. coli*.



*E.coli*



Determination of sequence of each clone

Analysis of species compositions comparing the sequences.



# Bacterial species dynamics during Asian dust event

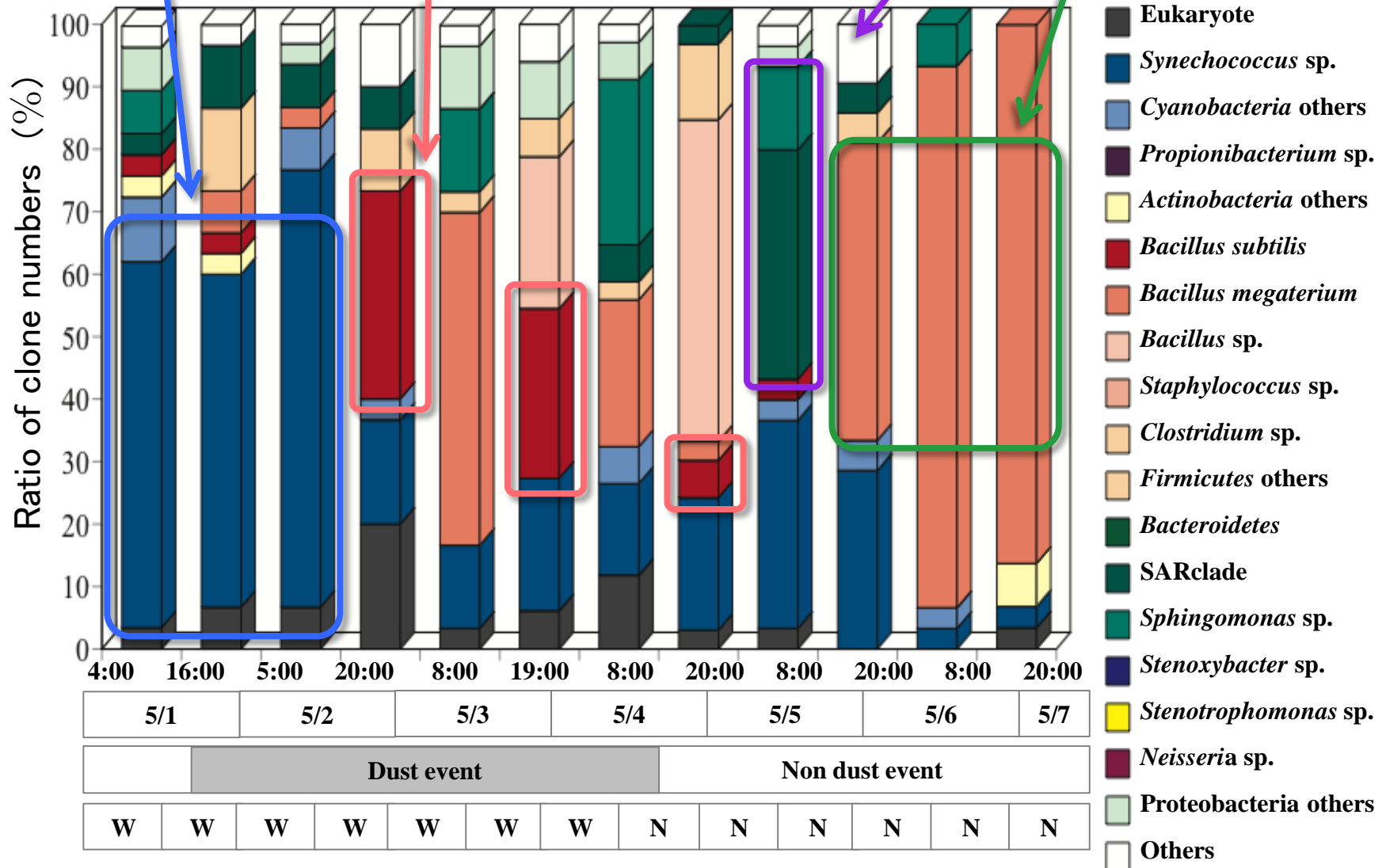
Marine cyanobacteria

Transported bacteria  
*B. subtilis*  
*Staphylococcus* sp.

Cold environmental species

Local origin

> 30 clones for each sample



A yellow arrow pointing to the right, with a black outline and a yellow fill. It has a tail with three vertical bars of increasing width. The text "Westerly wind" is written in blue inside the arrow.

Westerly  
wind

A yellow rounded rectangle with a black outline, containing the text "Direct sampling in atmosphere" in red.

Direct sampling  
in atmosphere

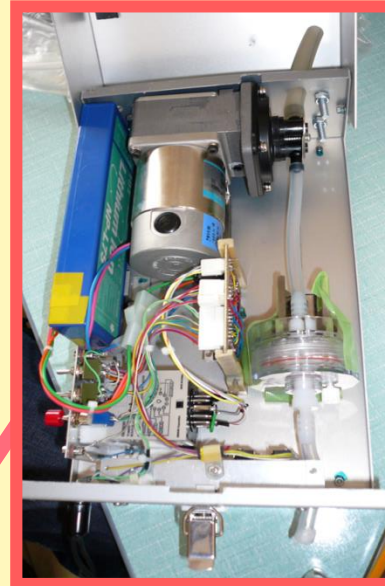
Avoid local  
contamination



# Aircraft

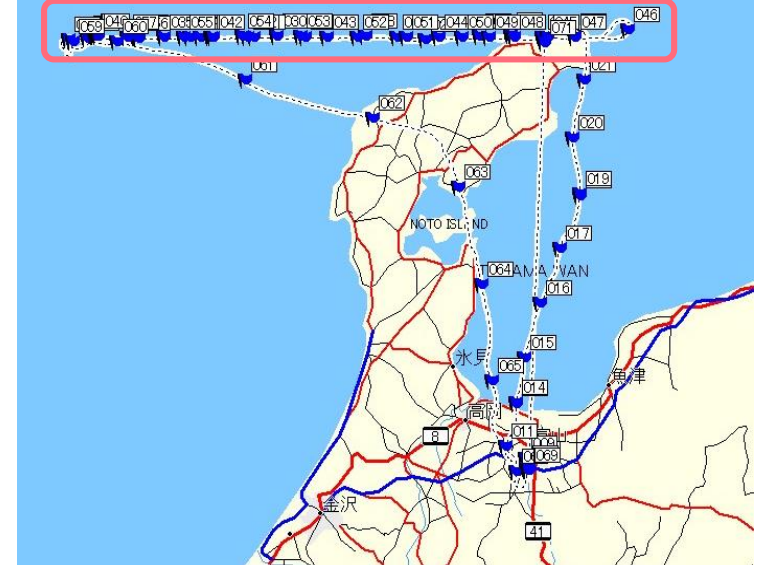


# Air sampling at 2000m ~ 3000m

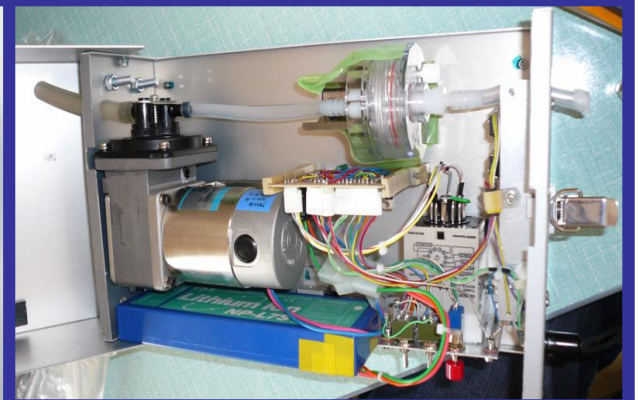


Collection on  
**0.2 $\mu$ m pore size polycarbonate filter** at rates of  
700 L/h using  
air pump.

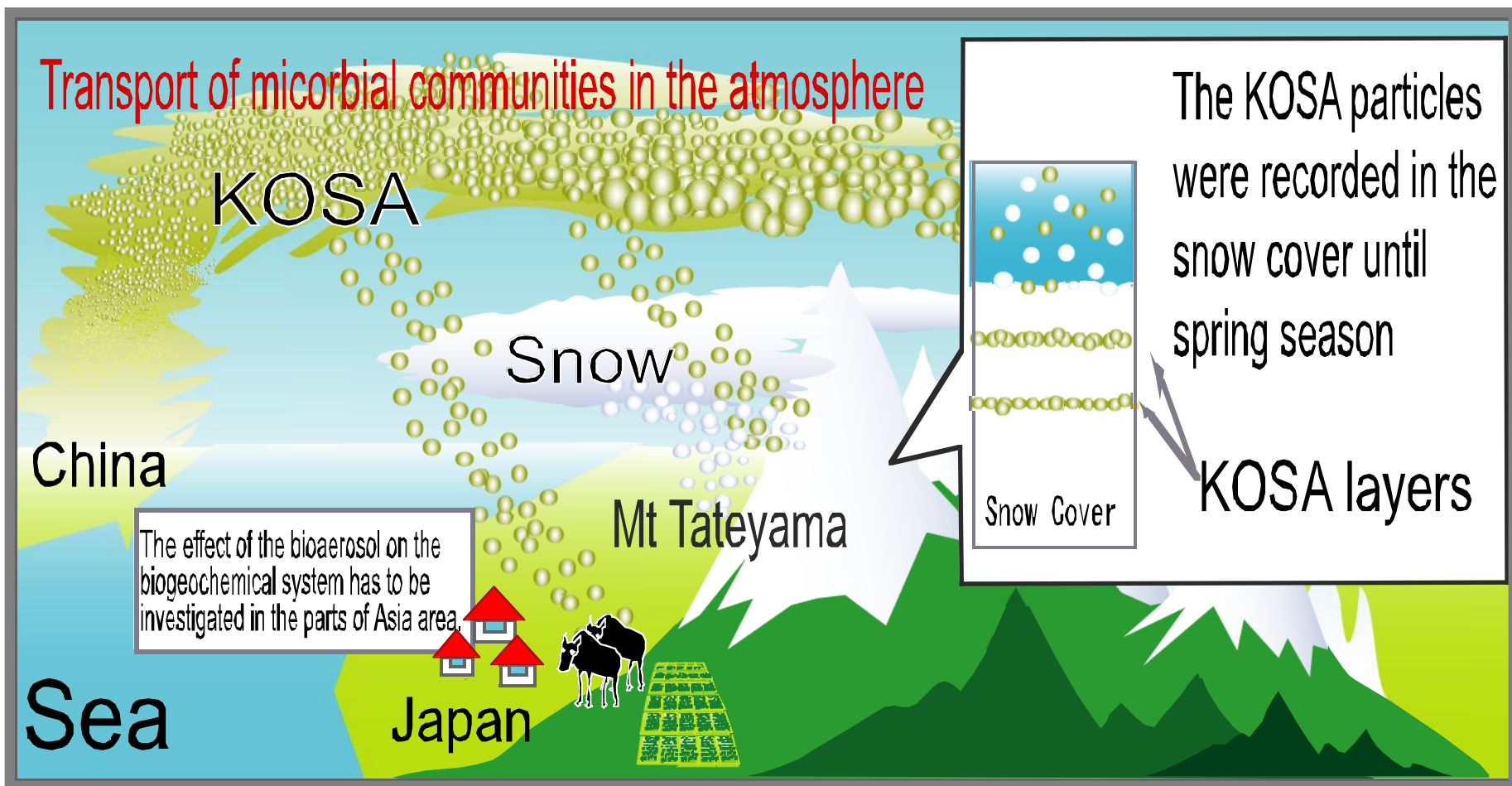
## Sampling course



Air sample collected at 800m  $\sim$  1000m



# Snow cover at Mt. Tateyama



The snow layers are expected to include high amounts of KOSA particles

# Snow cover sampling (Mt. Tateyama, Murododaira)

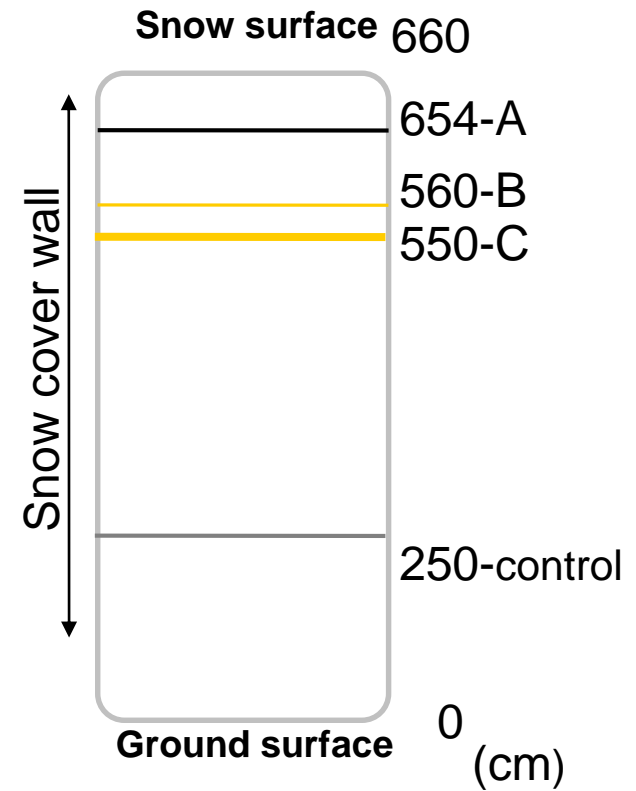


Murododaira, 2450m  
The snow cover at depth  
of 6m60cm from Fall to  
Sp

Sampling dates

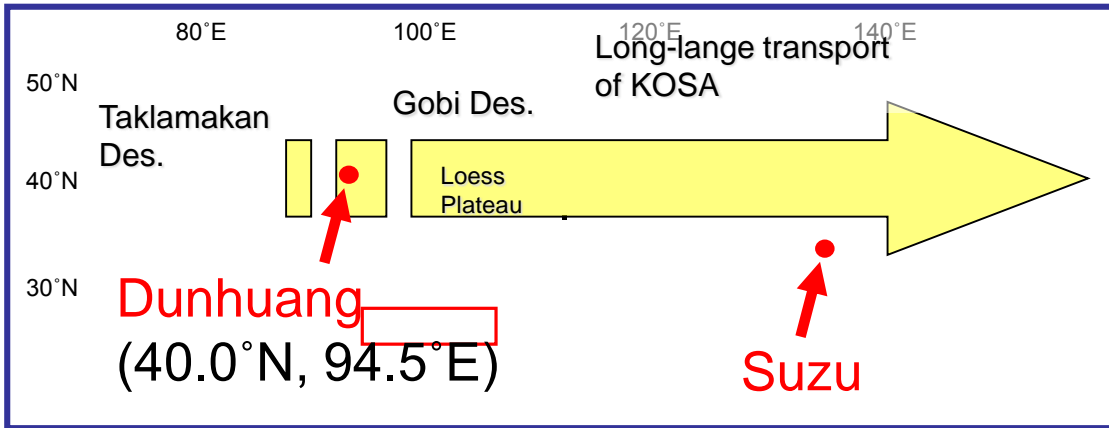
2008 April 16

2010 April 16

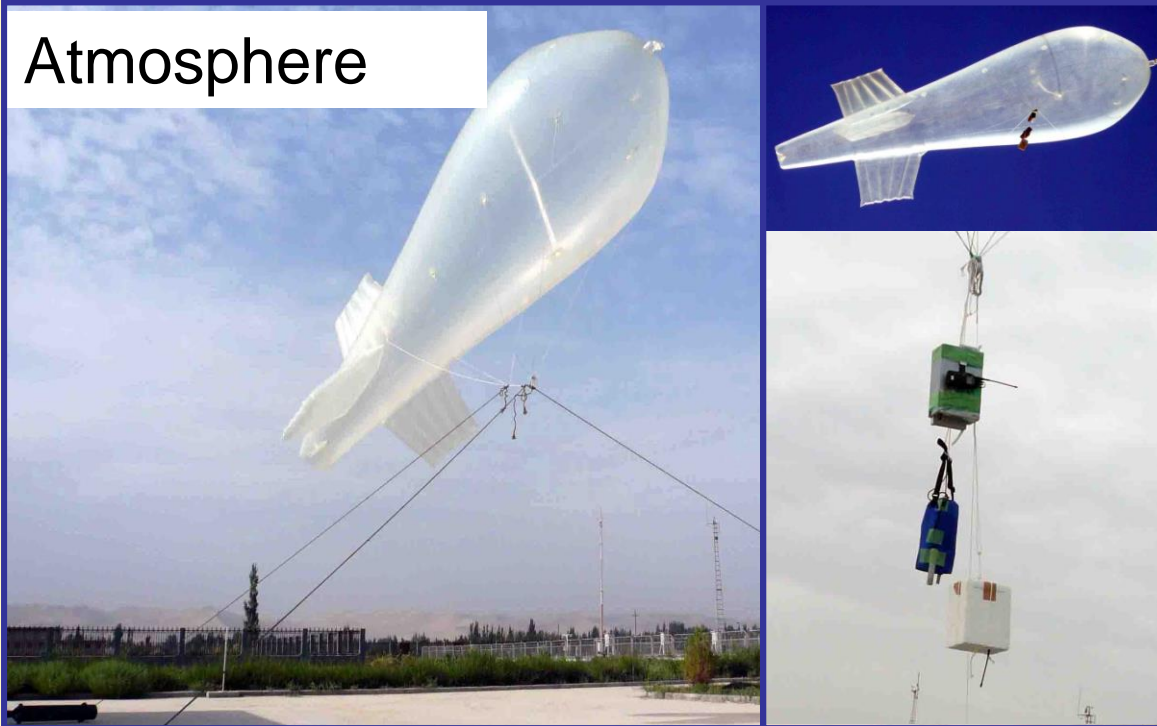


Layers B and C contained Ca at high concentrations of 3.3 mg/L and 1.2 mg/L, respectively, while Ca were not detected from the snow samples of control layer and Layer A.

# Balloon sampling (Dunhuang City, Suzu City)



## Atmosphere



Aerosol sample was collected at heights of 800 m using an air pump with  $0.2 \mu\text{m}$  membrane filter (200 m<sup>3</sup>)

Dunhuang 800 m

2007 August 17

13:15~14:15

Suzu 800m

2008 May 7

11:00~12:00



Balloon for high altitude



Ground sampling



# Air Sampling at 10m

---

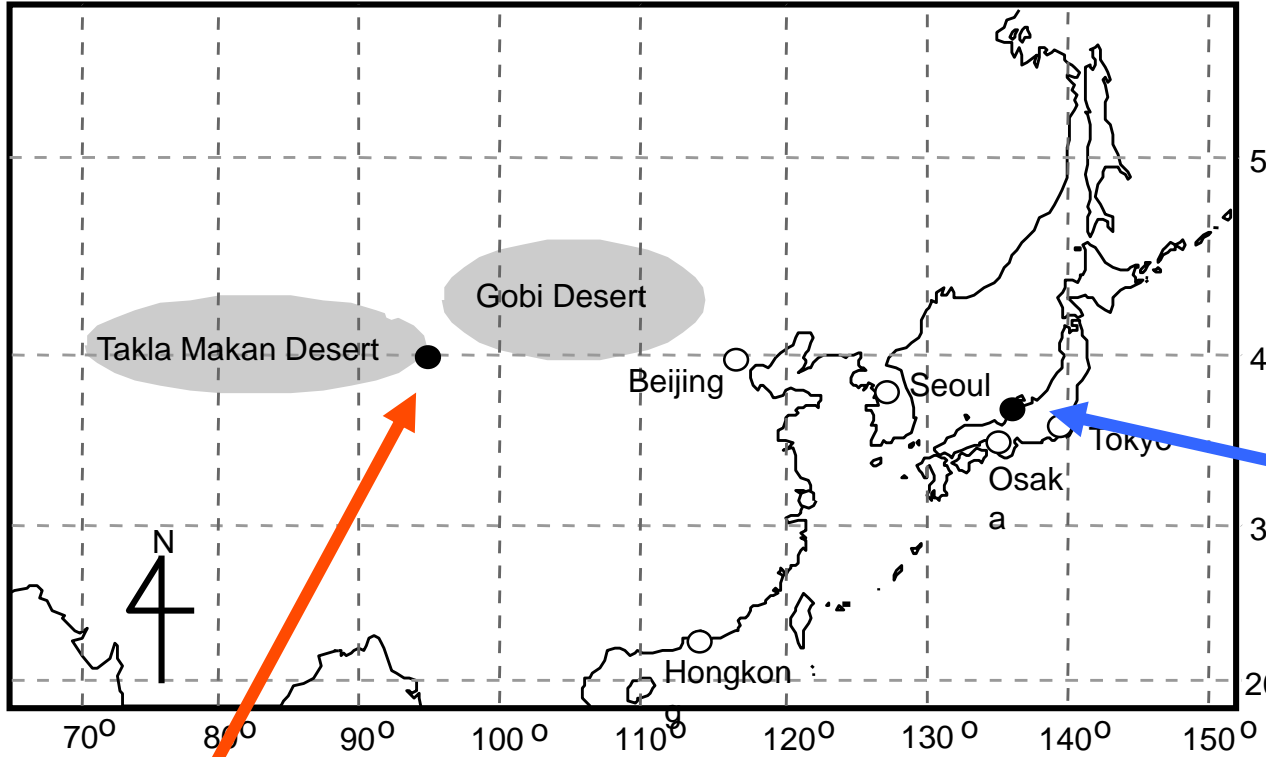
Metrological Department  
in Dunhuang city



# Experimental space



# Sampling in Dunhuang and Kanazawa



Dunhuang (Source)

Noto Peninsula (Arrival)

Aircraft 3000m



Baloon 1000m



Baloon 1000m



Ground 10m

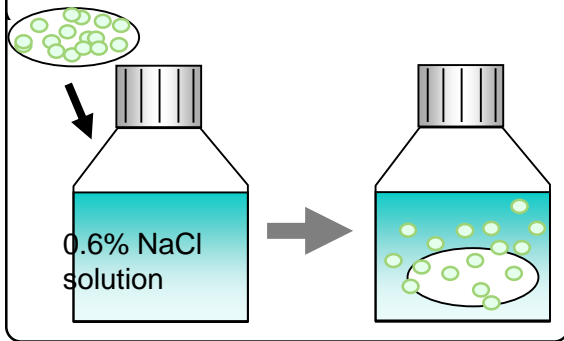


Ground 10m



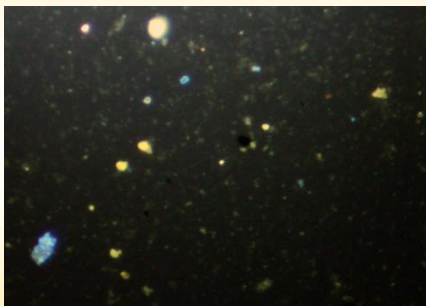
# Approach for analyzing bacterial communities

Bioaerosol on filter were washed by liquid solution of 0.6% NaCl solution.



Stain using DAPI

Microscopic observation

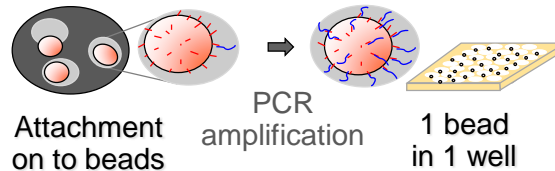


Extraction of genomic DNA

Culturable bacteria <1% → Bacterial gDNA is analyzed

PCR: Amplification of 16S rRNA genes

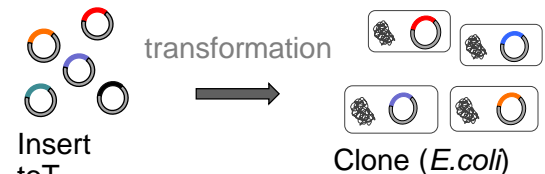
Pyrosequencing



>1000 reads (ca. 400bp) of each samples were determined using Roche 454.

Analysis of bacterial compositions

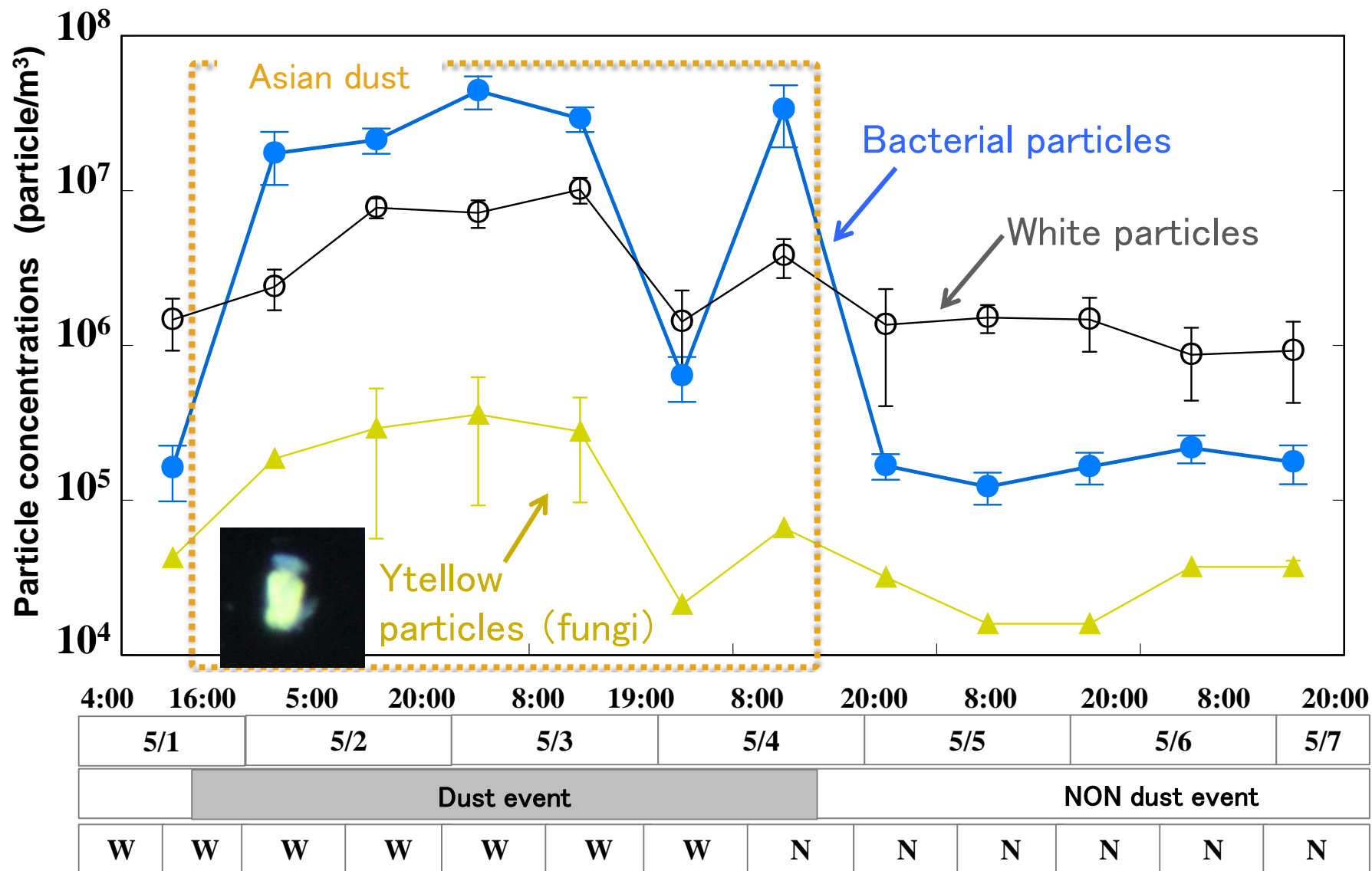
Clone library



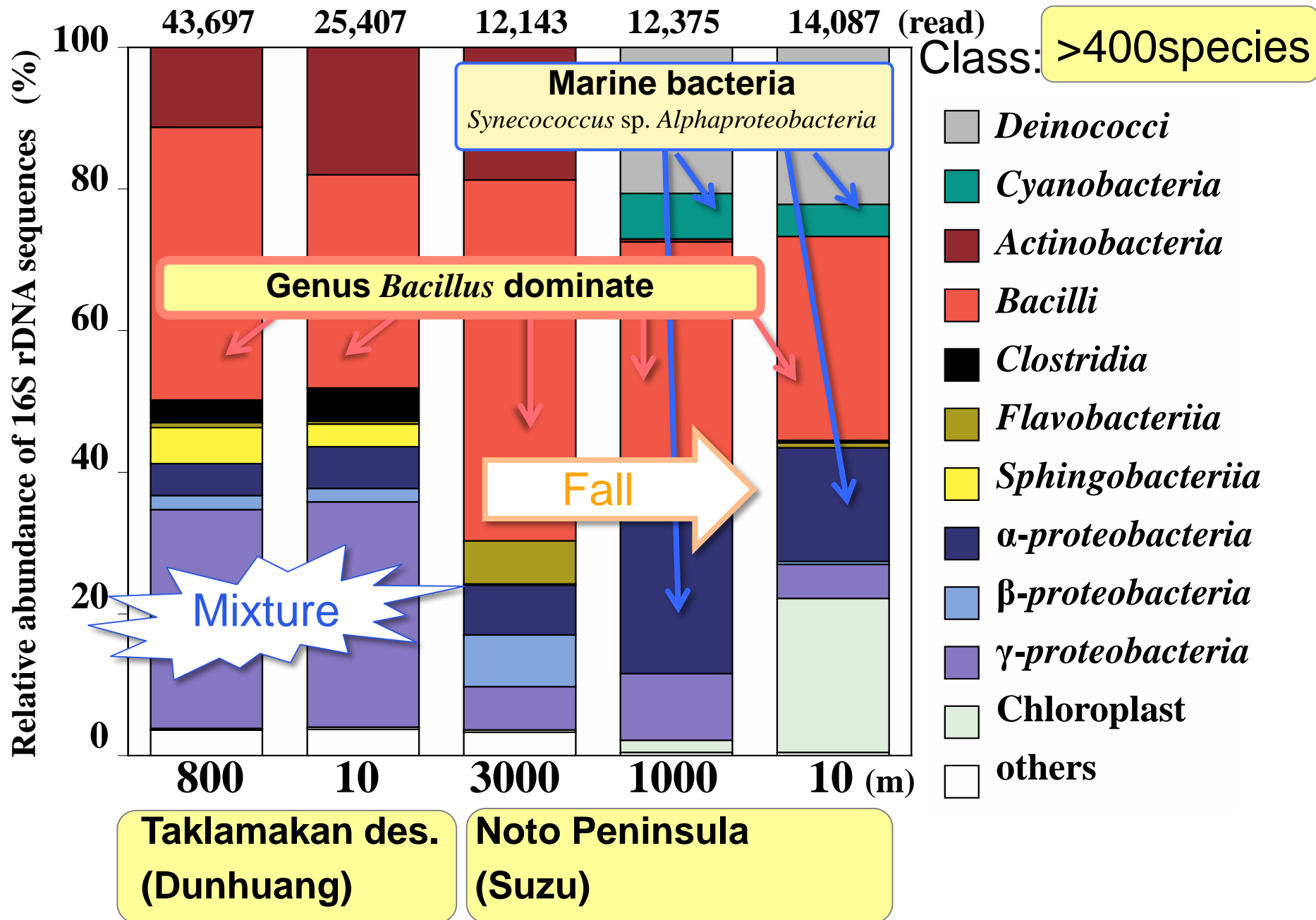
16S rDNA fragment (ca. 1,400bp) inserted in >50 clones were determined

Phylogenetic analysis

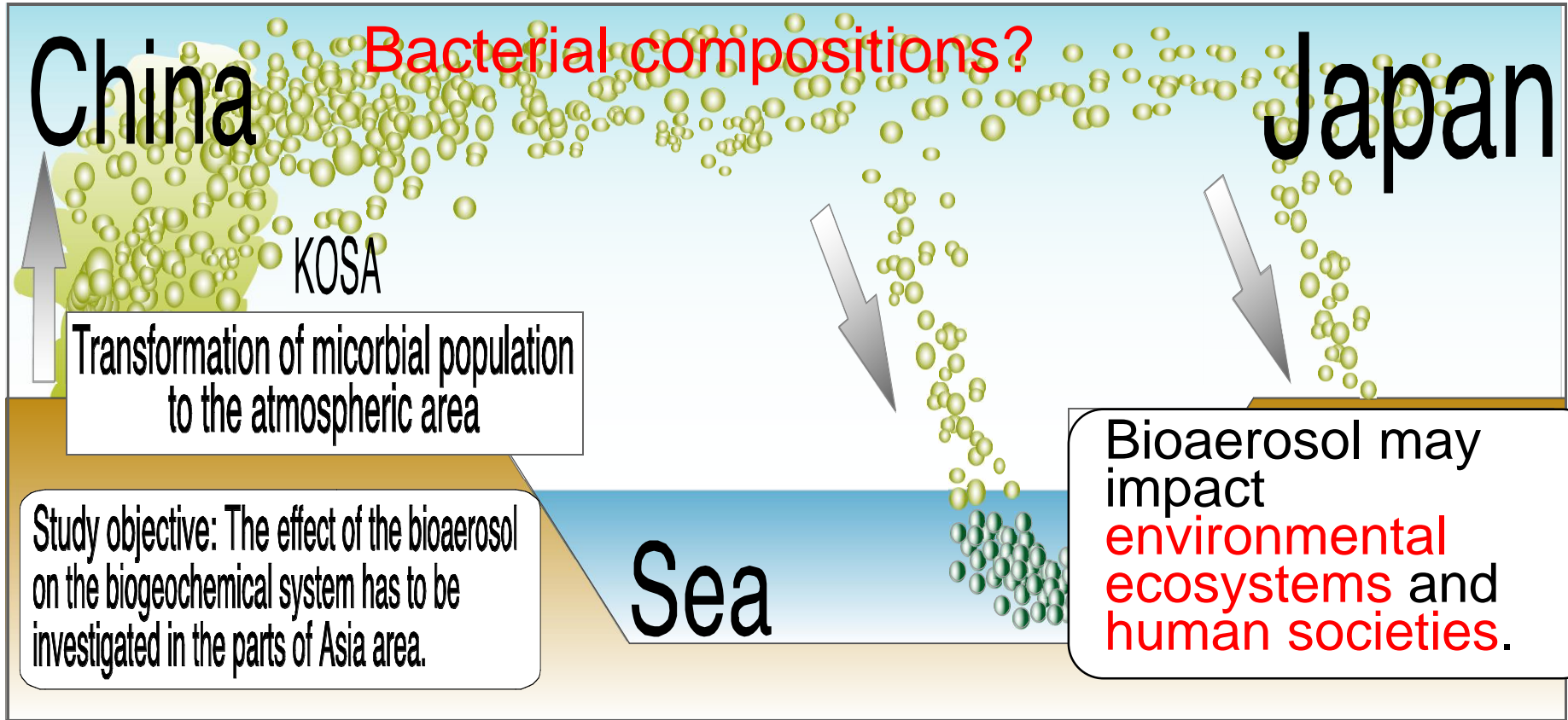
# Changes in particle in concentrations



# Bacterial structures in atmosphere over Dunhuang and Suzu

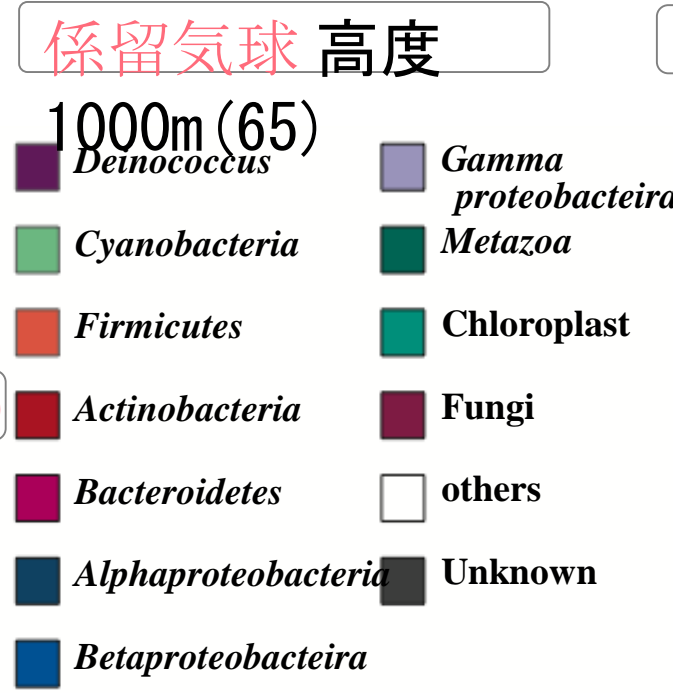
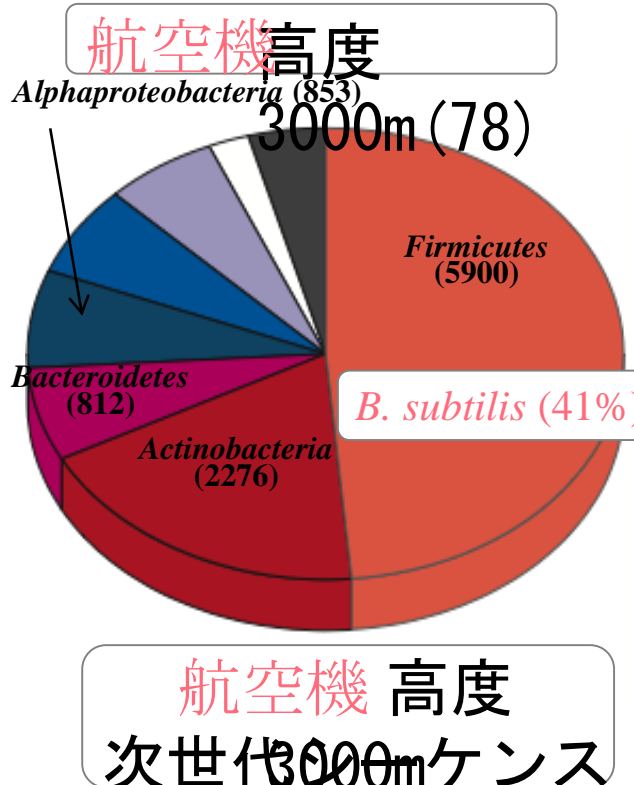
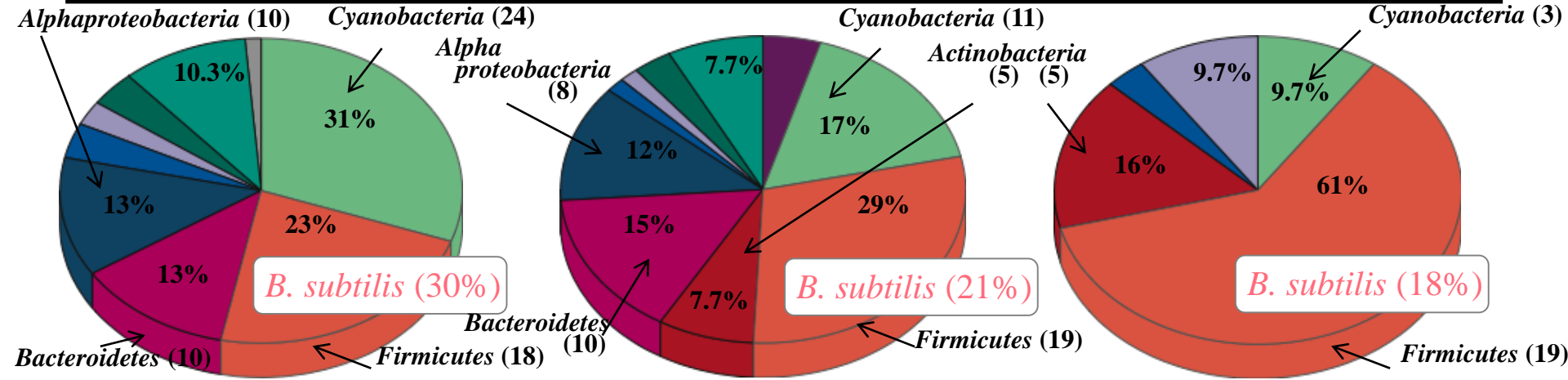


# Long-distance transport of Bioaerosol





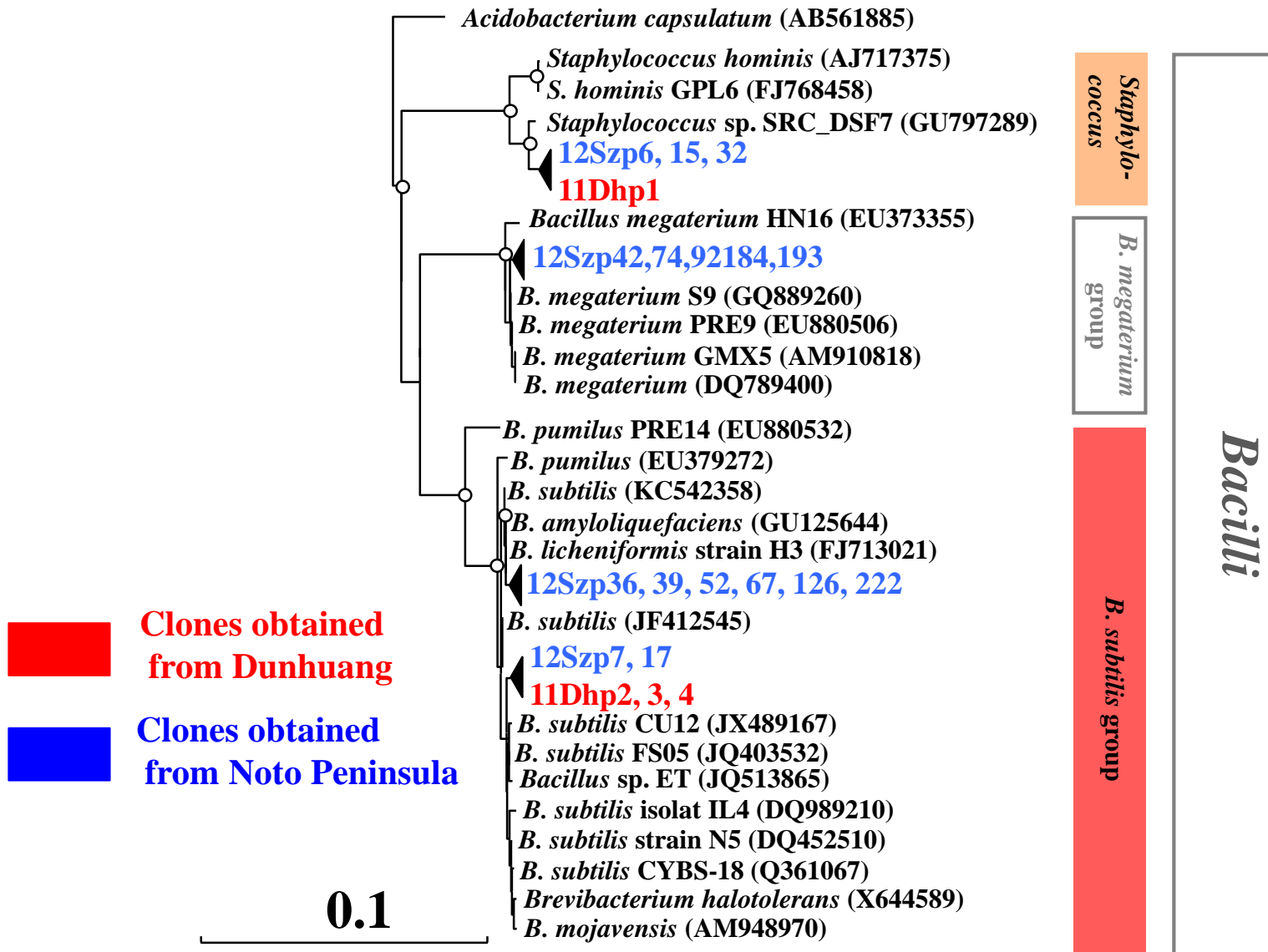
# 16S rDNA クローンを用いた細菌種組成の垂直分布の比較



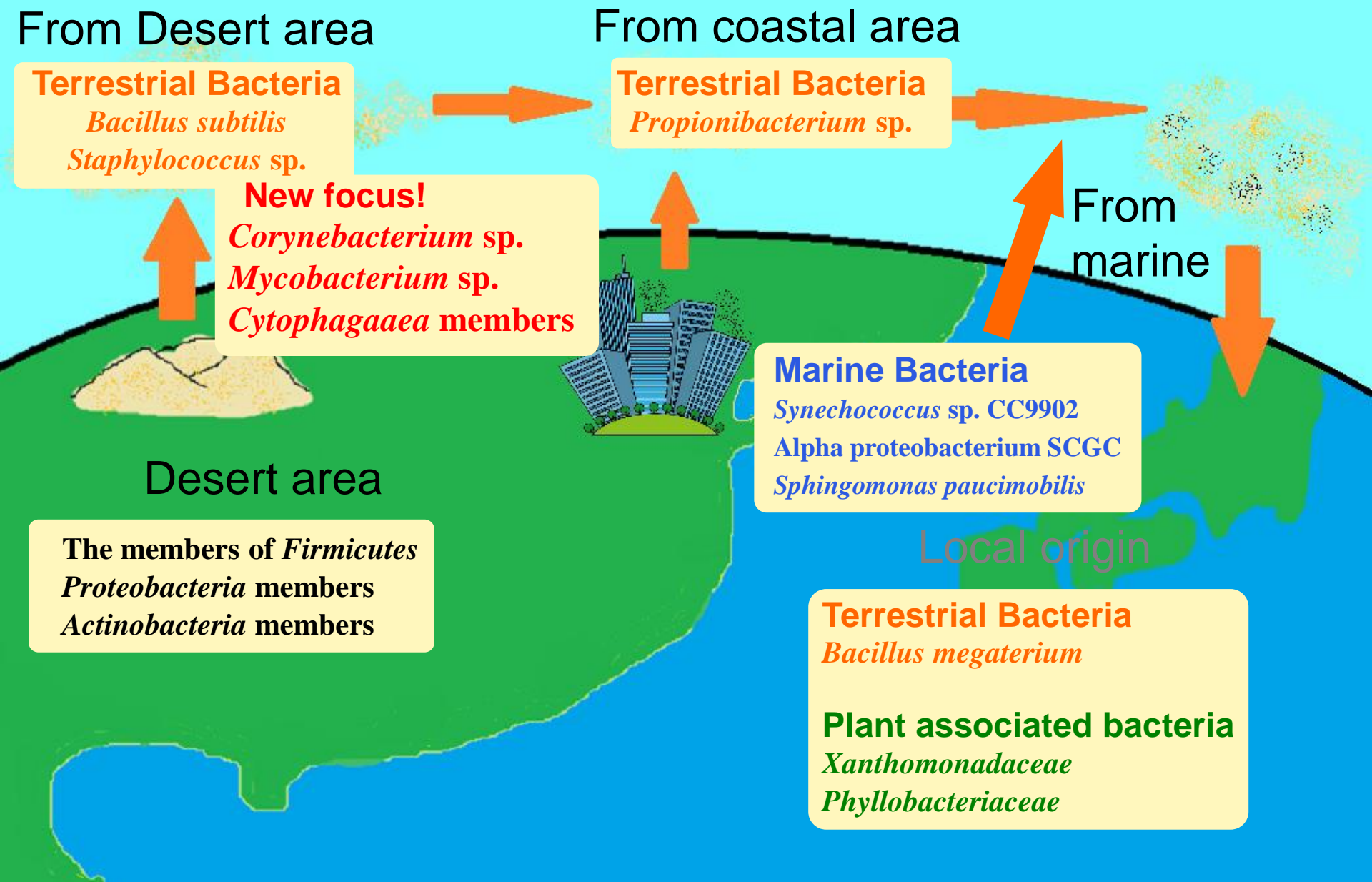
**地上調査 高度 10m (31)**

Alphaproteobacteria およびCyanobacteria は海洋起源であり、Firmicutesは土壌起源であると推察できる。ただし、*B. subtilis* は風送されてきた可能性がある。

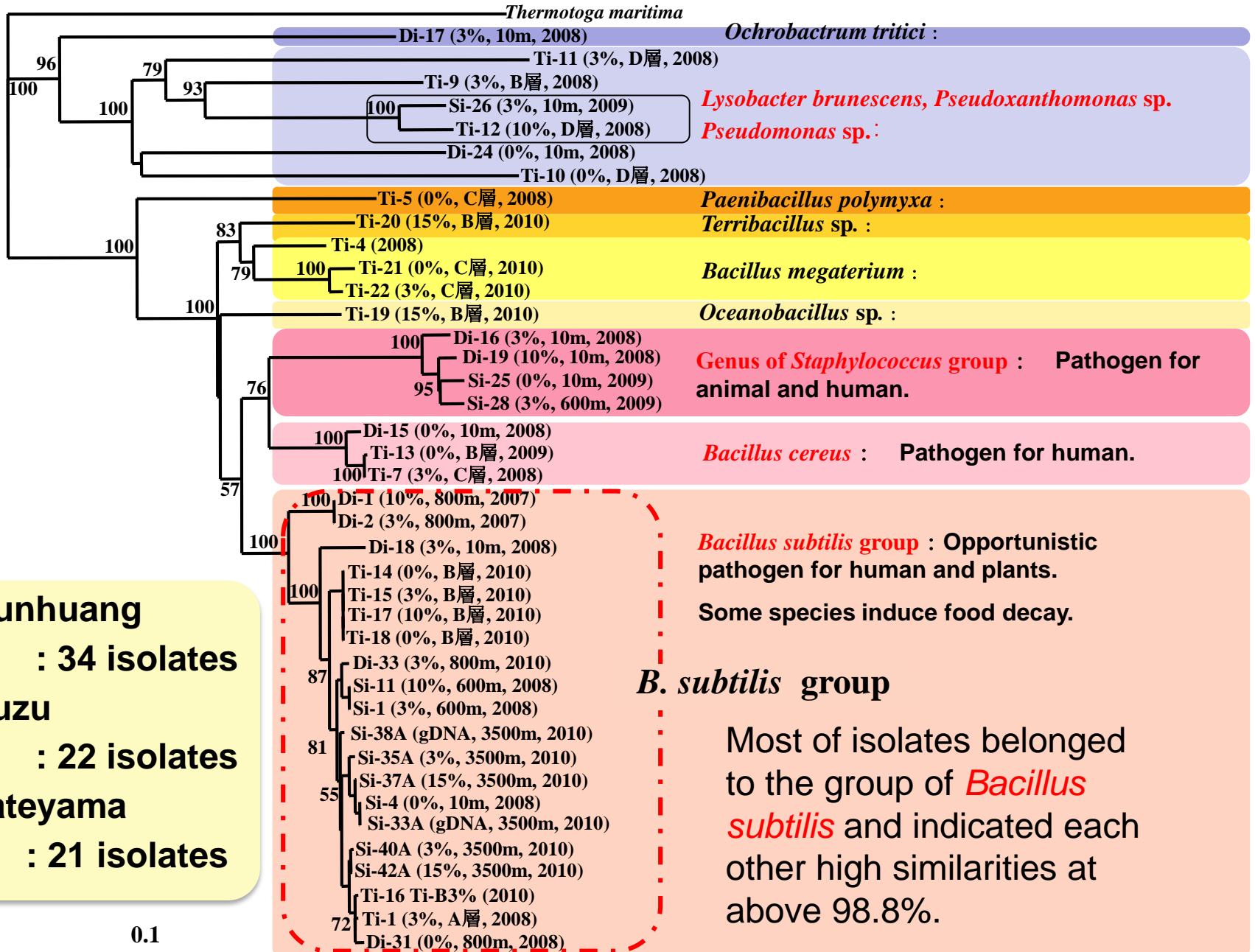
# Phylogenetic tree of *Bacilli*



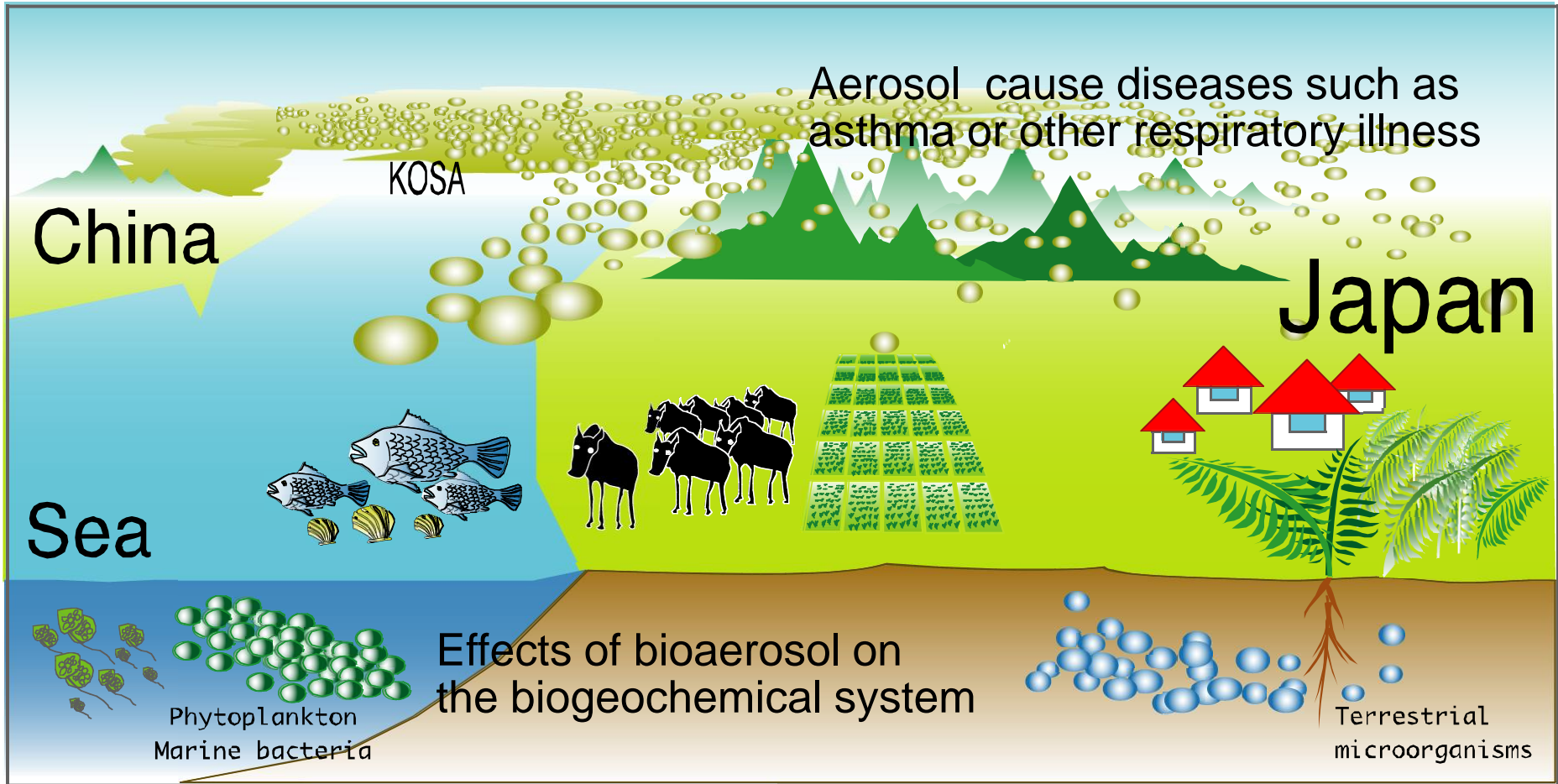
# Bacterial population should be focused



# Phylogenetic tree based on 16S rDNA sequences



# Negative effects of bioaerosols



Asian dust events carry some allergen, such as mineral particles, chemical compounds, and pathogen, in the atmospheric area.

# Positive effects of bioaerosols

Activation as  
Ice-nuclear for  
forming clouds

KOSA

**Climate**

China

Production of  
Japanese  
fermented foods

**Food**

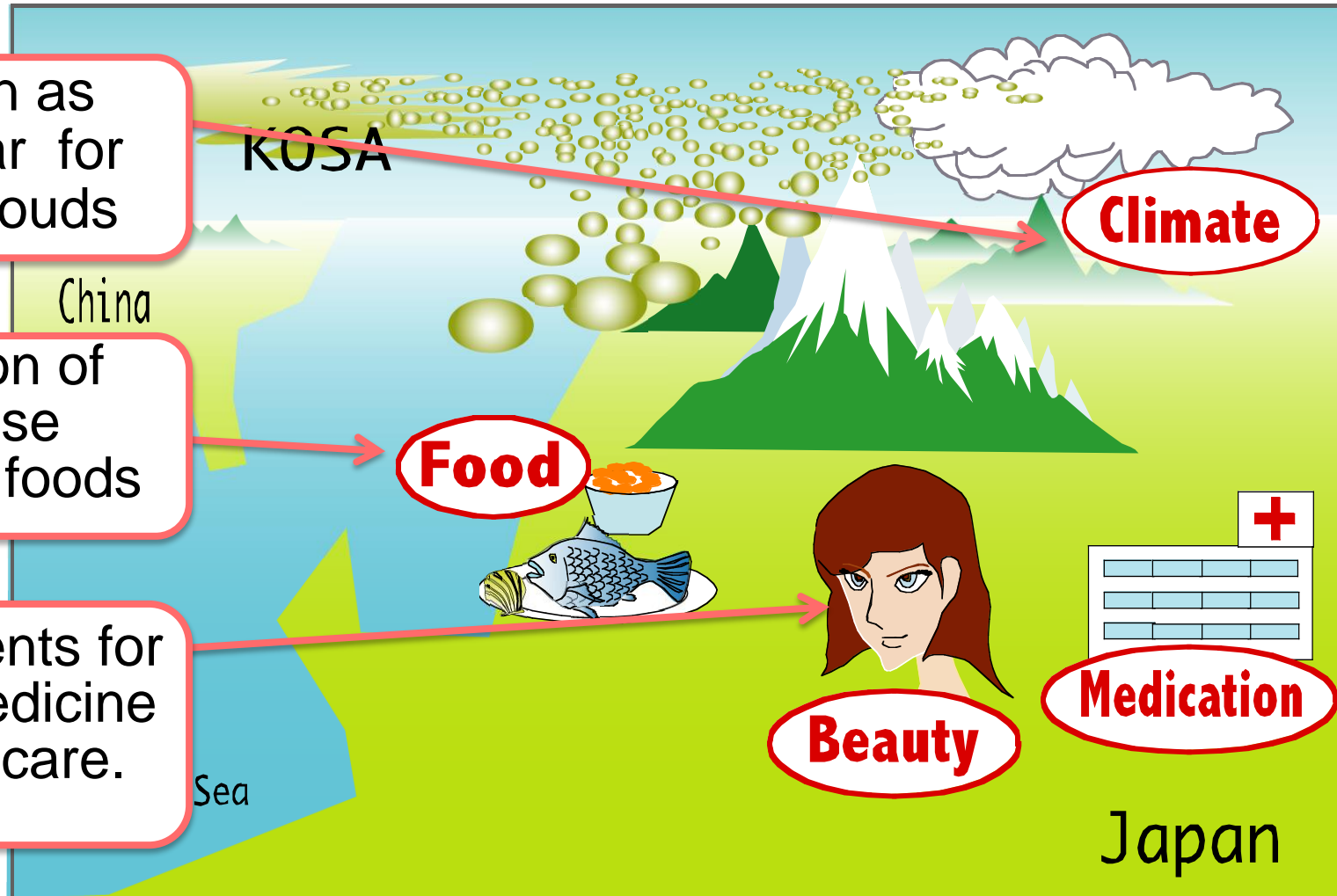
Developments for  
medical medicine  
and health care.

**Beauty**

**Medication**

Sea

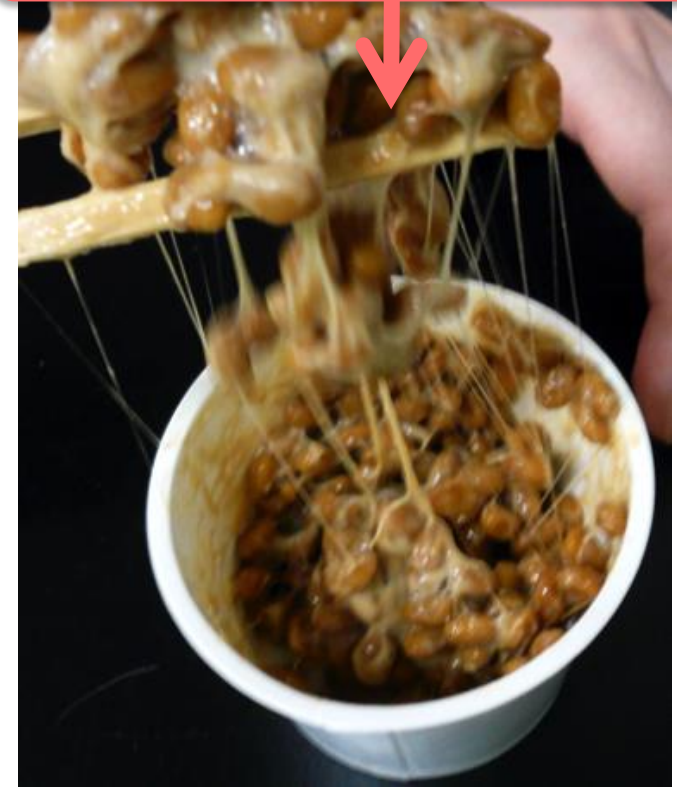
Japan



# Evaluation of Poly-gamma-glutamic acid

試料	寒天培地 NaCl濃度	スタータ液 NaCl濃度	納豆1gあたりの PGA重量(ug/g)
Si-41	3%	3%	460
	3%	10%	830
	10%	10%	620
Si-38	3%	3%	150
	3%	10%	100
	10%	10%	180
Si-37	3%	3%	130
	3%	10%	160
	10%	10%	260
Si-38 (製品試作)	3%	10%	120±8
Si-41 (製品試作)	3%	10%	190±14
金城納豆	-	-	180±34

Sticky and stringy caused by  
Poly-gamma-glutamic acid



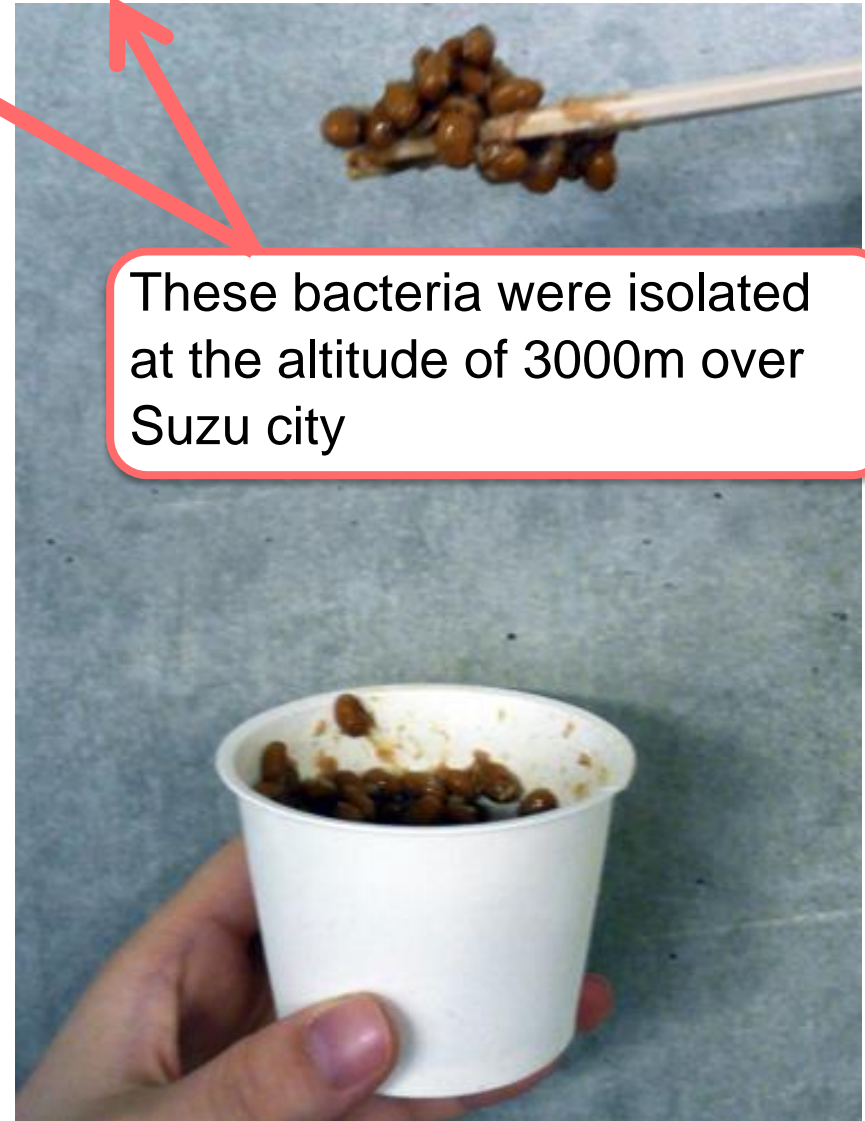
Amounts of Poly-gamma-glutamic acid included in natto products were different among the strains.

# Comparison of sticky among bacterial strains

Si-37 (33-38)



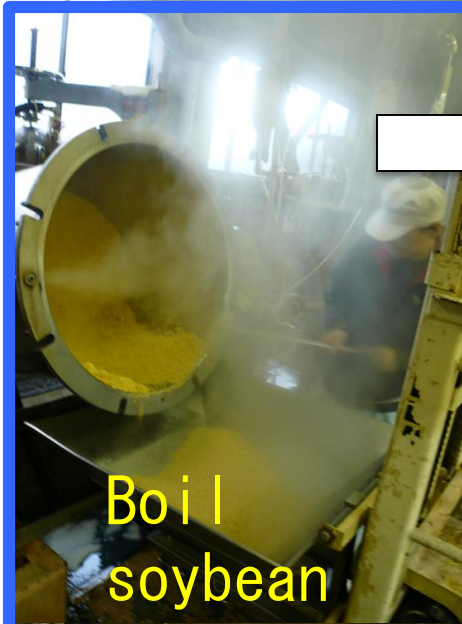
Si-39 (39-42)



These bacteria were isolated at the altitude of 3000m over Suzu city



# Natto producing with Kinjo Natto



# Japanese traditional health food “Natto”



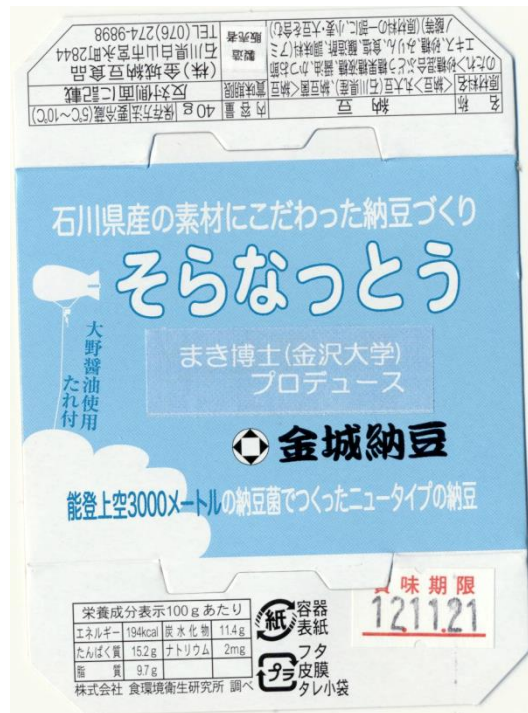
Location : Restaurant in Kanzawa Univ., Store in Ishikawa Prefecture in Japan, et al.

Start Date : From 10 July, 2012 (Natto day)

Price : 50 yen/1 piece, 100 yen/2 piece

Products : 5000 piece/month

# Japanese traditional health food “Natto”



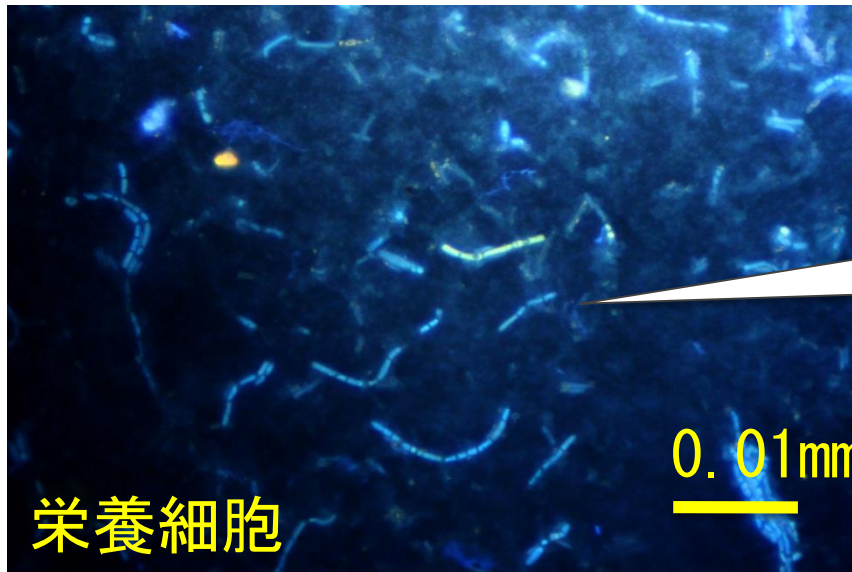
Location : Restaurant in Kanzawa Univ., Store in Ishikawa Prefecture in Japan, et al.

Start Date : From 10 July, 2012 (Natto day)

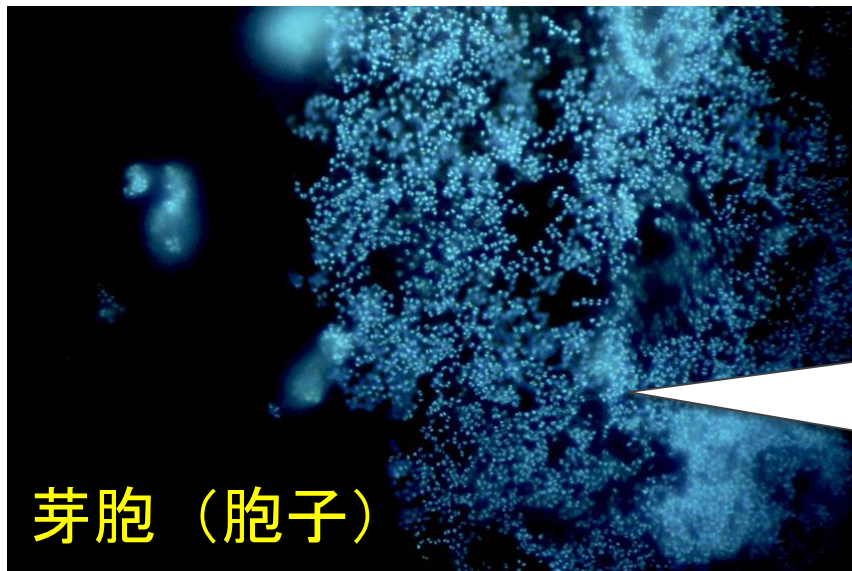
Price : 50 yen/1 piece, 100 yen/2 piece

Products : 5000 piece/month

# なぜ納豆菌が空を飛ぶのか？



増殖する時



乾燥に強く、  
熱 (80°C) にも  
耐える。

# Mushroom

A fluorescence micrograph showing the structure of Bjerkandera adusta hyphae. The image displays several long, thin, yellowish-green hyphae against a dark background. Some hyphae are straight, while others are curved or branched. The hyphae exhibit a granular texture. In the upper right corner, there is a cluster of more complex, interconnected structures, possibly representing a developing fruiting body or a dense network of hyphae. The overall appearance is that of a filamentous fungus.

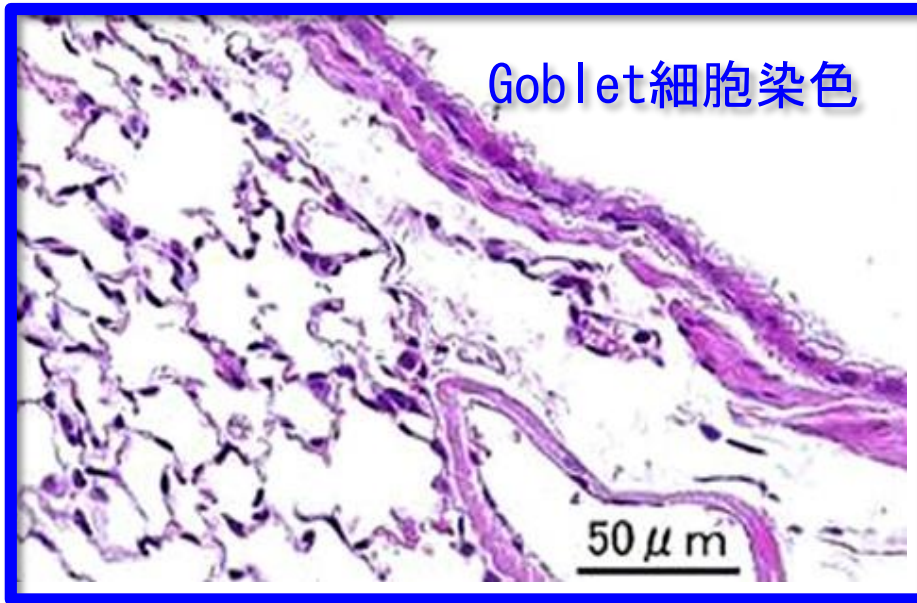
*Bjerkandera adusta*

10  $\mu\text{m}$

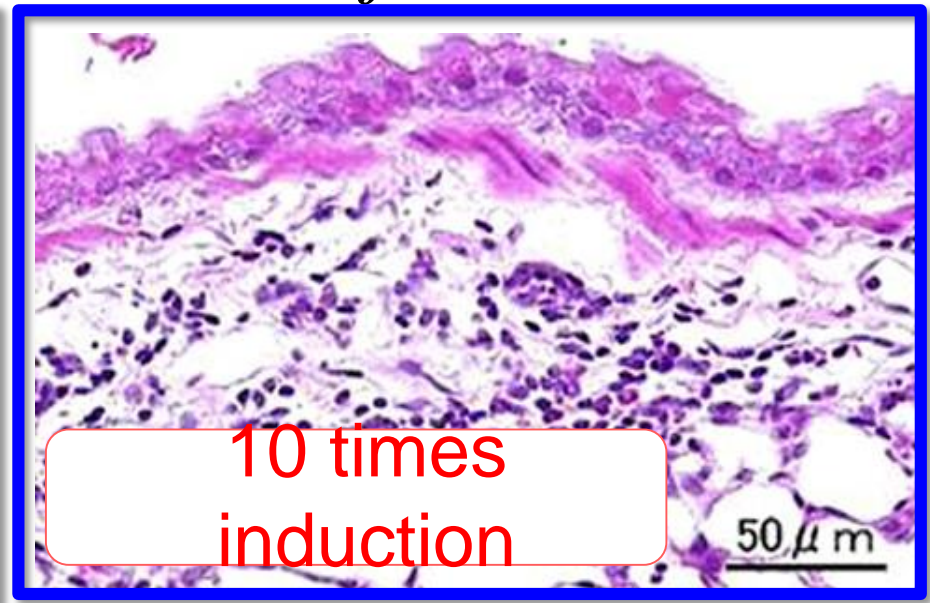
A horizontal yellow scale bar located in the bottom right corner of the image, used to indicate the magnification level of the micrograph.

# Induction of allergy

Dust particles



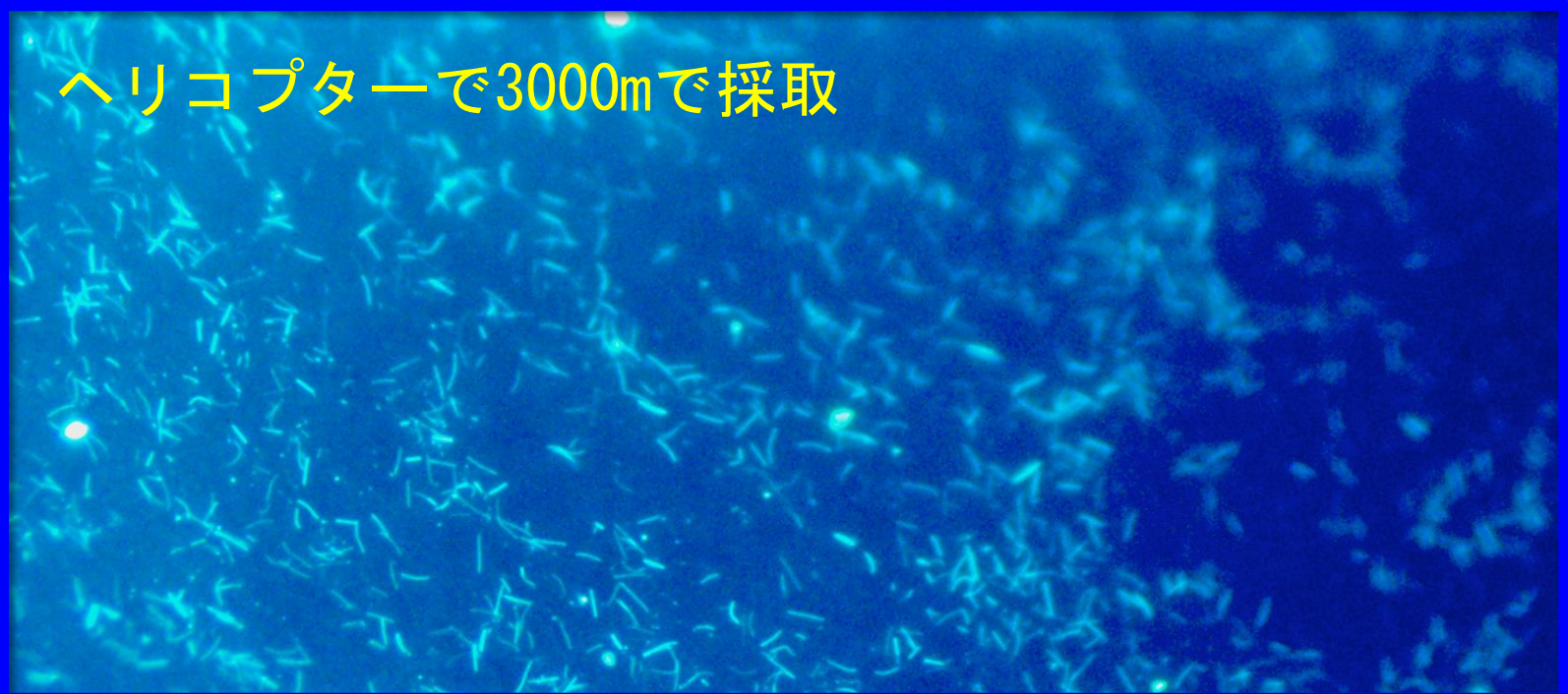
Dust particles  
+ *Bjerkandera*



マウスの気道上皮細胞

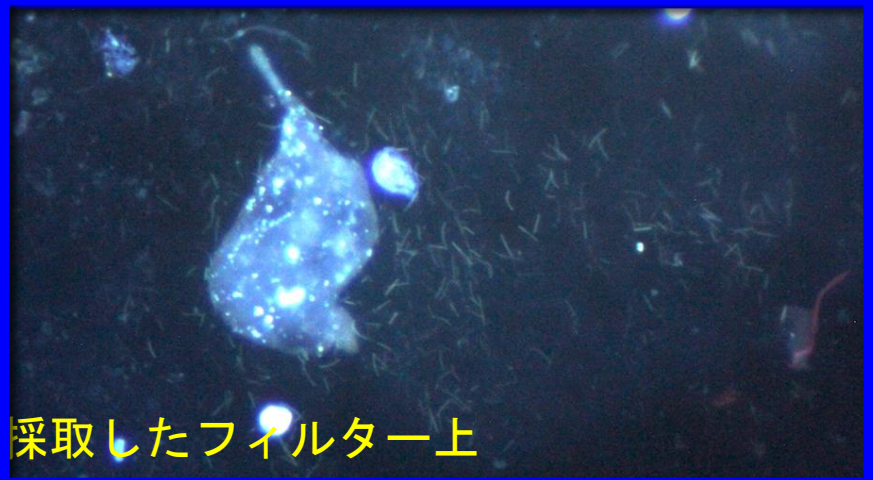
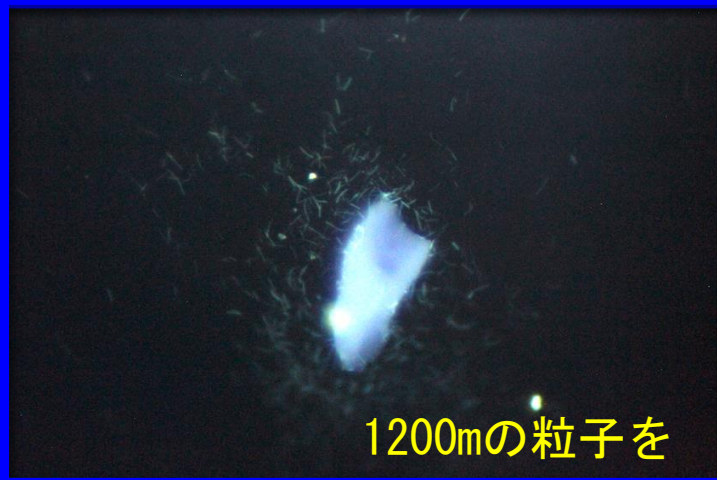
# 謎 3 大気中のラーメン状物質

ヘリコプターで3000mで採取



1200mの粒子を

採取したフィルター上



# 真菌のエアロゾル化

真菌株のエアロゾル粒子

10um

酷似

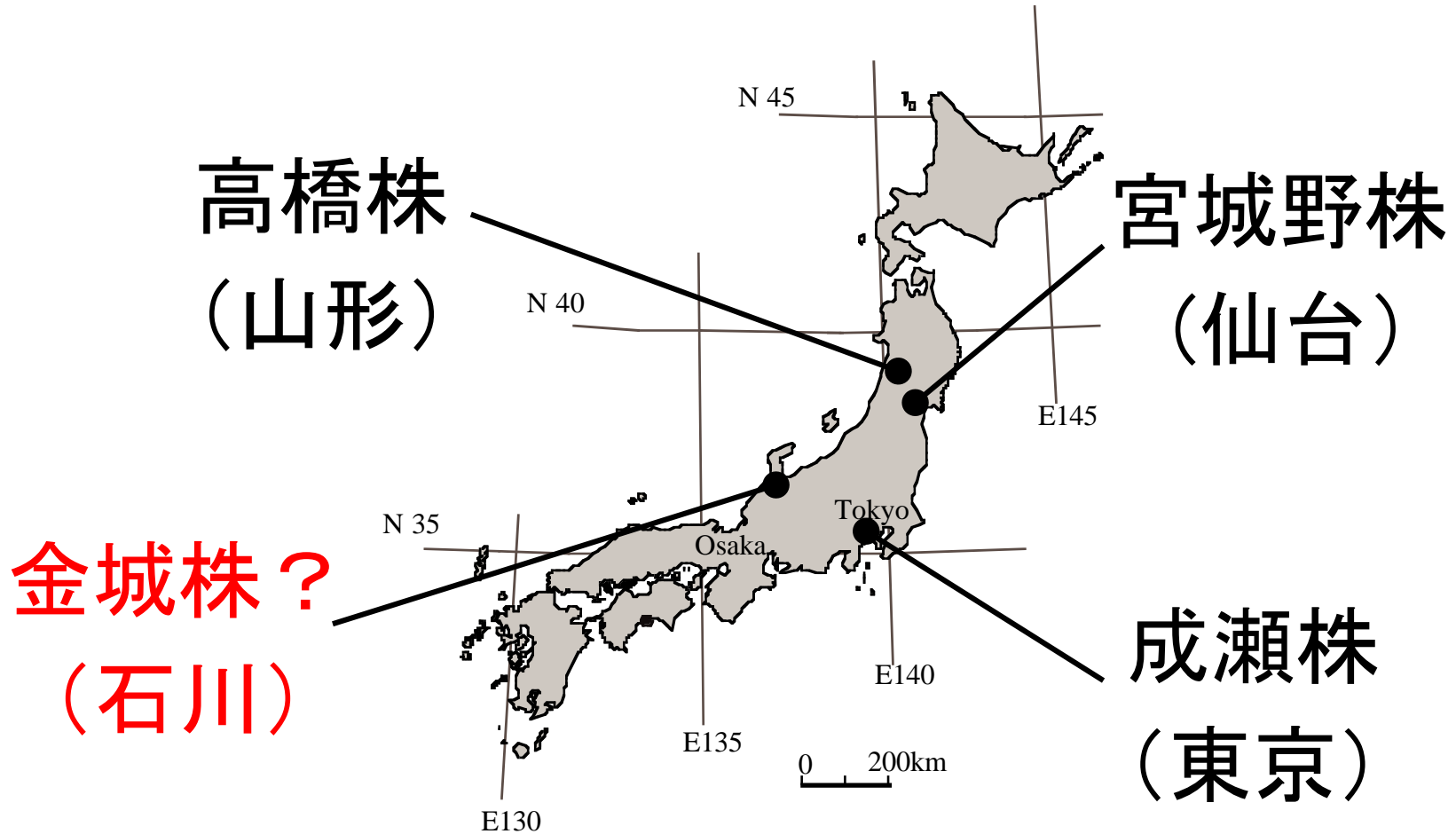
飛ぶ？

上空3000mで捕集した  
エアロゾル粒子

真菌株の培養細胞



# 第四の納豆菌株へ



# Bioaerosol & Food cultures

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## Natto

*Bacillus subtilis* ferments soybean. There is also natto food “豆鼓” in China.

## Shiokara

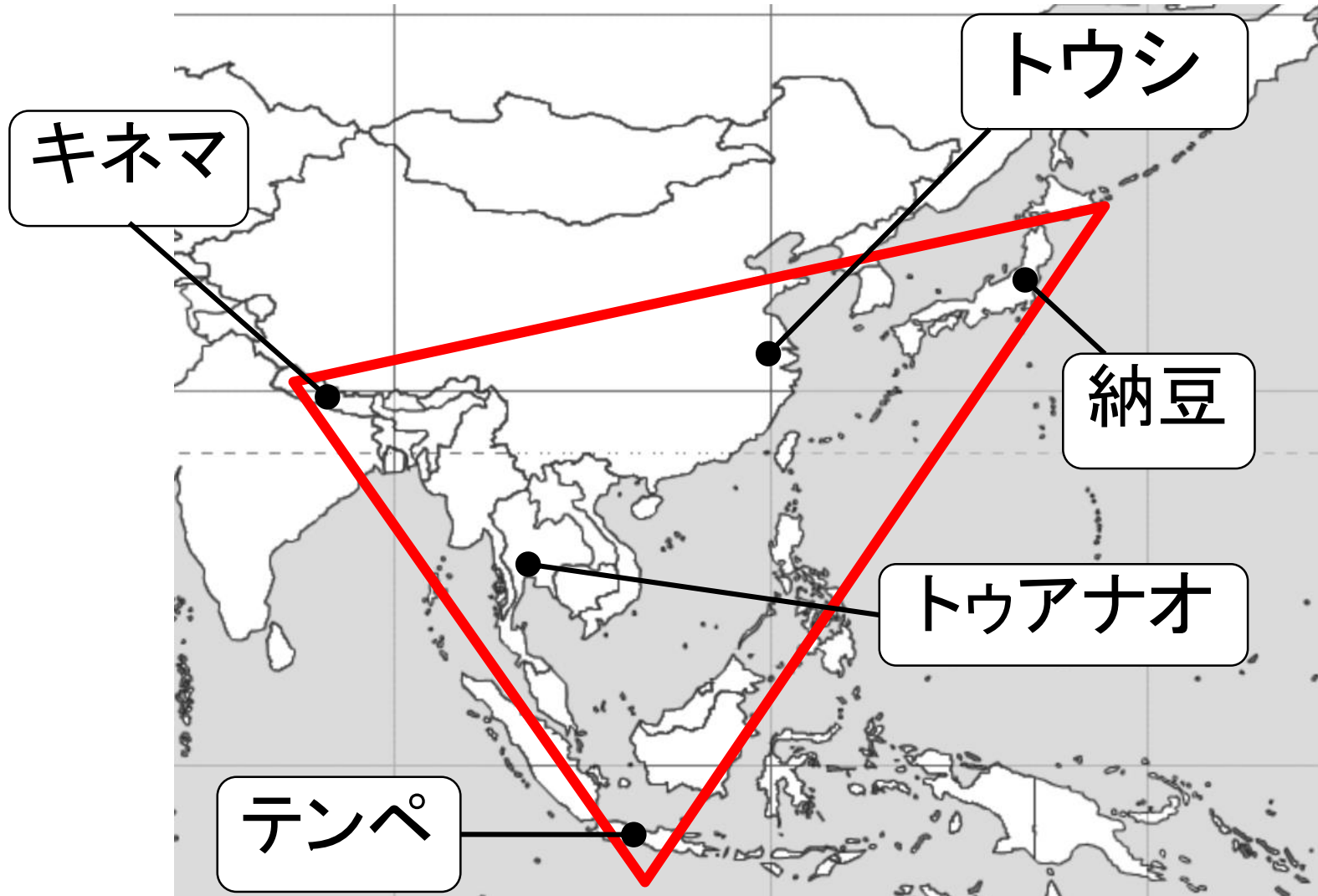
Components of squid are fermented by the autolysis, and the genus *Staphylococcus* related to the make of Umami.

## Fish Sauce (Shotturu, Ishiru)

Protease produced by halophilic *Bacillus* and Ocean *Pseudomonas* induce the mature (Soujou).

Atmospheric microorganisms (Continental origin) may support to the fermentation of Japanese traditional ferment foods.

# 納豆トライアングル



In this lecture

We discussed new style concept of life ,  
especially for bioaerosols  
and

Possible system of balloon borne lidar is  
discussed as the observational tool  
desired for bioaerosol research.

Thank you for your attention!