# Variations in the structure of airborne bacterial communities in a downwind area during an Asian dust (Kosa) event



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Title:

**Variations in the structure of airborne bacterial communities in a downwind area during an Asian dust (Kosa) event**

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**Abstract**

Asian dust (Kosa) events transport airborne microorganisms that significantly impact biological ecosystems, human health, and ice-cloud formation in downwind areas. However, the composition and population dynamics of airborne bacteria have rarely been investigated in downwind areas during Kosa events. In this study, air samplings were sequentially performed at the top of a 10-m high building within the Kosa event arrival area (Kanazawa city, Japan) from May 1 to May 7, 2011, during a Kosa event. The particle concentrations of bacterial cells and mineral particles was ten-fold higher during the Kosa event than on non-Kosa event days. A 16S ribosomal DNA clone library prepared from the air samples primarily contained sequences from three phyla: *Cyanobacteria*, *Firmicutes*, and *Alphaproteobacteria*. The clones from *Cyanobacteria* were mainly from a marine type of *Synechococcus* species that was dominant during the first phase of the Kosa event and was continuously detected throughout the Kosa event. The clones from *Alphaproteobacteria* were mainly detected at the initial and final periods of the Kosa event, and phylogenetic analysis showed that their sequences clustered with those from a marine bacterial clade (the SAR clade) and *Sphingomonas* spp. During the middle of the Kosa event, the *Firmicutes* species *Bacillus subtilis* and *Bacillus pumilus* were predominant; these species are known to be predominat in the atmosphere above the Chinese desert, which is the source of the dust during Kosa events. The clones obtained after the Kosa event had finished were mainly from *Bacillus megaterium*, which is thought to originate from local terrestrial areas. Our results suggest that airborne bacterial communities at the ground level in areas affected

by Kosa events change their species compositions during a Kosa event toward those containing terrestrial and pelagic bacteria transported from the Sea of Japan and the continental area of China by the Kosa event.

**Keywords:** Asian dust, airborne, bioaerosol

## 1. Introduction

Asian dust (Kosa) events from the deserts of northern China, including the Gobi and Taklamakan deserts, pass over the Sea of Japan and disperse mineral-dust aerosol over the East Asian region via westerly winds (Duce et al., 1980, Iwasaka et al., 1983). The mineral-dust particles are associated with microbial particles commonly called "bioaerosols," which include viruses, bacteria, and fungi (Jones and Harrison, 2004, Jaenicke, 2005, Prospero et al., 2005). Airborne microorganisms carried by dust events increase the allergen burden, causing an increased incidence of asthma (Ichinose et al., 2005) and contributing to the dispersion of diseases such as Kawasaki disease in humans (Rodó et al., 2011) and rust diseases in plants (Brown and Hovmøller, 2002). Moreover, bioaerosols are thought to influence atmospheric processes by participating in atmospheric chemical reactions and cloud particle formation (Pratt et al., 2009, Creamean et al., 2013).

The dynamics of airborne bacteria in downwind areas during Kosa events need to be elucidated in order to understand the characteristics of bacterial communities that are transported long distances and influence downwind ecosystems and climates. The size and composition of airborne bacterial communities at high altitudes above Kosa dust deposition areas such as Beijing (Li et al., 2010), Osaka (Yamaguchi et al., 2012), Noto Peninsula (Maki et al., 2013), and the North American mountains (Smith et al., 2012) varied significantly depending on the Kosa event studied. Investigations of airborne microbial dynamics at the ground level in China and Korea indicated that soma bacterial species from *Firmicutes* were predominant during Kosa events (Jeon et al., 2011).

However, there are a few reports investigating bacterial dynamics at the ground level in Japan when the dust particles that have passed through China and Korea have traversed the Sea of Japan. Dust particles reaching Japan are reported to change their chemical composition when passing over Chinese industrial area and the Sea of Japan. Thus, the chemical and biological characteristics of dust particles in Japan are expected to differ from the dust particles collected from Korea and China, and, in fact, it was reported that bacterial abundance and viabilities at the ground level in Japan dynamically changed during a Kosa events (Hara and Zhang, 2012). However, the diversity and structure dynamics of airborne bacteria have yet to be investigated in a populated area in Japan during a Kosa event.

To investigate the population dynamics of airborne bacteria in Japan during a Kosa event, we collected air samples at the ground surface in Kanazawa city from May 1 to May 7, 2011, during a long-term of Kosa event. We determined the abundance of bioaerosols in the air samples by microscopic observation using a fluorescence staining technique. The composition of the bacterial species in the air samples was analyzed using clone-library analysis targeting bacterial 16S ribosomal RNA genes (16S rDNA).

## 2. Materials and Methods

### 2.1. Sampling

Aerosol samplings were performed in a coastal city, Kanazawa (36.33°N, 136.39°E), on the south area of the Sea of Japan from 7:00 JST on May 1 to 8:00 JST on May 7., 2011, when a long-term Kosa event occurred (May 1 to May 4). Kanazawa

city is located on the northern coast of the Hokuriku region in Japan, where the aerosols from continental areas arrive directly from the Sea of Japan and aerosol contamination from Japan can be eliminated. The sampling system was placed on a 10-m high platform (located at Kanzawa University). Air samples (520 L) were collected using sterilized polycarbonate filters (0.22 μm pore size; Whatman, Tokyo, Japan) with a sterilized filter holder using an air pump. Four each sample, two filters were used continuously for 12 h; the filters were changed every 12 h. In total, 12 air samples were obtained during the sampling period from the morning of May 1 to the evening of May 7, named Sample 1 to Sample 12. Of the two filters used to collect each sample, one filter was used to determine the abundance of bioaerosols by microscopic observation, and the other one was stored at –80°C before extraction of genomic DNA for the analysis of bacterial species compositions.

### 2.2. Characteristics and trajectories of air masses during the sampling period

Air quality and atmospheric data at the ground surface were obtained from the meteorological observatories of the Japan Meteorological Agency in Kanazawa city and Wajima city, which are located 10 and 50 km, respectively, from the sampling site. Environmental data were collected every hour. Information regarding atmospheric environmental factors, such as temperature, precipitation, relative humidity, wind velocity, dew point temperature, and steam pressure were obtained for analyzing air-mass dynamics (Table S1).

To track the transport pathways of air masses, 72-h backward trajectories were calculated using the National Oceanic and Atmospheric Administration Hybrid Single

Particle Lagrangian Integrated Trajectory (HYSPLIT) model (http://www.arl.noaa.gov/HYSPLIT.php). The location of the backward trajectory start point was the sampling location for this study (36.33°N, 136.39°E) with altitudes of 10, 1,000, and 3,000 m above the ground for estimating the accurate trajectories of air masses. The depolarization ratios of particles below 3,000 m on May 1 and May 7, 2011 were measured using light detection and ranging (lidar) at Toyama city (http://www-lidar.nies.go.jp/).

### 2.3. Microscopic analysis of particle abundance

Within 2 h of sampling, 1 mL of sterilized water with paraformaldehyde at a final concentration of 1% was added to one of the filters to fix the aerosols. After a 1-h incubation, the filter was stained with 4 ,6-diamidino-2-phenylindole (DAPI) at a final concentration of 0.5 μg $mL^{-1}$ for 15 min (Porter and Feig, 1980). Next, the filter was placed on a slide in a drop of low-fluorescence immersion oil. A second drop of oil was added, and a coverslip was placed on top. The prepared slides were then observed using an epifluorescence microscope (Olympus, Tokyo, Japan) equipped with a ultraviolet excitation system. A filter transect was scanned, and mineral particles (white particles), yellow particles and bacterial cells on the filter transect were counted. The detection limit of aerosols was below $5 \times 10^3$ particles $m^{-3}$ of air.

### 2.4. Cloning analysis targeting 16S rDNA sequences

Aerosols were washed off the filters by shaking with 5 mL of Tris-ethylenediaminetetraacetic acid buffer. After washing, the aerosols were collected by

centrifugation at 20,000 × *g* for 5 min. Genomic DNA (gDNA) was extracted from the bacterial cell pellets using sodium dodecyl sulfate, proteinase K, and lysozyme, as described previously (Maki et al., 2008). gDNA was purified by phenol-chloroform extraction, followed by chloroform extraction and ethanol precipitation. Fragments of 16S rDNA (approximately 1,450 bp) were amplified from the extracted gDNA by polymerase chain reaction (PCR) using the following oligonucleotide primers: 27F, 5′-AGA GTT TGA TCM TGG CTC AG-3′; 1492R, 5′-GGY TAC CTT GTT ACG ACT T-3′ (Maidak et al., 1997). Thermal cycling was performed using the Program Temp Control System PC-700 under the following conditions: 30 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 2 min. PCR amplicons corresponding to 16S rDNA fragments were purified by phenol-chloroform extraction followed by chloroform extraction and ethanol precipitation; they were then cloned into *Escherichia coli* using a commercially available vector with a TA Cloning Kit (Invitrogen, CA, USA) according to the manufacturer's protocol. More than 50 clones were obtained for each sample, and the sequences were determined using a Dye Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems, CA, USA) and an ABI Prism 373A DNA Sequencer according to the manufacturer's recommended protocols. The M13 forward primer was used as the sequencing primer.

The amplicon sequences were compared against the DNA Data Bank of Japan (DDBJ) using Basic Local Alignment Search Tool (BLAST) to analyze bacterial species compositions. All sequences without chimeras were assigned as operational taxonomic units (OTUs) based on bacterial species with more than 97% similarities. The coverage of the 16S rDNA library was calculated using the formula [1 – (n/N)], where n is the

number of OTUs represented by one clone and N is the total number of clones (Good 1953). The full sequences of the dominant clones were determined using the M13 reverse primer. Phylogenetic trees including representative sequences were constructed according to the neighbor-joining algorithm using TreeViewPPC (Saitou and Nei 1987).

### 2.5. Accession numbers

DDBJ accession numbers for the 16S rDNA sequences determined in this study are shown in Table 1.

## 3. Results

### 3.1. Environmental conditions

During the sampling period, the relative humidity varied randomly around 40%, and the temperature from May 2 to May 5, 2011 fluctuated at lower values, below 20 ºC, than on May 1 and from May 6 to May 7 (Table S1). Some precipitation occurred on May 1, suggesting that a cyclonic flow originating from the western North Pacific or from the East China Sea contributed to rainfall in Kanazawa city on May 1. From the evening of May 1 to the morning on May 7, the days were sunny and calm.

Analyses of air-mass backward trajectories revealed four variations from May 1 to May 7, 2011 (Fig. S1). The air mass on May 1, 2011 originated from the North Pacific Ocean and passed the southern or western parts of Japan to Kanazawa city (Fig. S1a). In contrast, the air mass between May 2 and May 3 came from the desert area of the Asian continent and passed over the industrial area in China and across the Sea of Japan (Fig.

S1b). From May 4 to May 5, the air mass was carried from the northern of the Sea of Japan or Korea Peninsula to Kanazawa city for 3 days with low-level transport over Korea and Japan (Fig. S1c). The air mass from May 6 to May 7 had a similar transport pattern to that of May 1 and came from the southern or western parts of Japan (Fig. S1d). Changes in aerosol transportation are primarily controlled by the prevailing air flowing from China.

According to lidar measurements at Toyama city, Japan, the depolarization ratio at the ground surface was significantly increased from the evening of May 1 to the evening on May 4; in addition, trajectory analysis indicated that the air mass during the period from May 1 to May 4 originated from west. These observations indicated that a long-term Kosa event occurred during this time around the Hokuriku area (Fig. S2). In this study, the period from the evening of May 1 to the evening on May 4 is defined as the Kosa event.

### 3.2. Microscopic observation of aerosols

When DAPI staining of aerosol particles collected at 10 m in Kanazawa city was performed on the sampling filter, the aerosols comprised both separate and aggregated particles. White-blue self-fluorescent particles, which were mineral particles, exhibited relatively large sizes, with diameters ranging from 0.2 μm to 100 μm. Yellow fluorescent particles, potentially organic matter, were observed to range from 0.2 μm to 10 μm in diameter. DAPI-stained bacteria were observed as coccoid and bacilli-like particles with a diameter of <1.0 μm and bright-blue fluorescence. These thee types of particles formed aggregates ranging from 2.0 μm to 100 μm in diameter. This indicates

that bacterial particles attached to large particles were transported through the atmosphere.

The total density of bacterial cells in air samples increased from $7.5 \times 10^4$ particles $m^{-3}$ to $2.0 \times 10^7$ particles $m^{-3}$ during the Kosa event and decreased to $7.7 \times 10^4$ particles $m^{-3}$ on non-Kosa event days (Fig. 1a). White-blue fluorescent particles with diameters ranging from 0.2 μm to 5.0 μm significantly increased to $4.5 \times 10^6$ particles $m^{-3}$ during the Kosa event and decreased to $10^5$ particles $m^{-3}$ after the Kosa event finished (Fig. 1b, c). White-blue particles of >5.0 μm diameters also increased to $7.4 \times 10^4$ particles $m^{-3}$ during the Kosa event but were less than $10^3$ particles $m^{-3}$ on non-Kosa event days (Fig. 1d). Yellow fluorescent particles from 0.2 μm to 5.0 μm in diameter fluctuated from concentrations of more than $2.4 \times 10^5$ particles $m^{-3}$ to $10^4$ particles $m^{-3}$ during non-Kosa event days (Fig. 1b, c). Yellow fluorescent particles of >5.0 μm diameter peaked at approximately $4.3 \times 10^4$ particles $m^{-3}$ during the Kosa event and were undetectable on non-Kosa event days (Fig 1d). The concentration of aggregated particles ranging from 0.2 μm to 5.0 μm in diameter increased to more than $1.4 \times 10^5$ particles $m^{-3}$ during the Kosa event but maintained values in the order of $10^3$ particles $m^{-3}$ on non-Kosa event days. Larger aggregated particles of >5.0 μm diameter significantly increased to $9.8 \times 10^4$ particles $m^{-3}$ during the Kosa event, decreasing to below limited detection on non-Kosa event days and increasing to $2.2 \times 10^4$ particles $m^{-3}$ at the non-Kosa event days May 6 and May 7.

### 3.3. Dynamics of 16S rDNA clone libraries

The 16S rDNA fragments in the air samples were amplified by PCR using primers

targeting eubacterial 16S rDNA. The PCR amplicons were cloned into *E. coli*, and a total of 402 clones including eubacterial 16S rDNA fragments were obtained from the 12 samples. Sequences of the 16S rDNA clones indicated that the airborne bacterial populations were composed of several bacterial species (Table 1). Reasonable coverage ranging from 80% to 93% was obtained, indicating that the majority of the airborne bacteria was represented in the libraries. Most of the majority of phylotypes recovered from the air samples belonged to the phyla *Cyanobacteria, Proteobacteria,* and *Firmicutes,* which are typically well represented in 16S rDNA clone libraries generated from terrestrial and marine environments. Only a few bacterial sequences were affiliated with *Actinobacteria* or *Acidobacteria*. The bacterial compositions of the clone libraries showed significant dynamics during the sampling period (Fig. 2). Only eight of the total 403 sequenced clones could not be affiliated with any known bacterial group.

Of the sequenced clones derived from all 12 samples, 27% were from *Cyanobacteria* (Table 1) and formed two distinct groups in the marine type cluster of the genus *Synechococcus* in the phylogenetic tree (Fig. 3). One group was composed of clones detected from Kosa event samples (Samples 3 and 5; Fig. 2). Sequences from this group clustered with the coastal and ocean *Synechococcus* spp. found in warm areas and were closely related to strains previously isolated from the Sea of Japan. In contrast, the sequences of the other group were detected in every sample and were mainly related to a coastal *Synechococcus* species found in cold environments.

More than 46.5% of the clones were from *Firmicutes* (Fig. 2), almost all of which were related to members from the genera *Bacillus* and *Staphylococcus* (>99.7% similarity). The complete 16S rDNA sequences of the *Bacillus* species had high

similarities (>99.7%) with *Bacillus subtilis*, *Bacillus pumilus* and *Bacillus megaterium* (Fig. 4). Some of the *B. subtilis* and *B. pumilus* clones appeared specifically at night during the Kosa event (Samples 4 and 6) and were closely related with isolates detected at high altitudes above Suzu city during a Kosa event (Maki et al. 2010) and above Taklamakan Desert (Maki et al. 2008). Other clones belonging to the genus *Bacillus* were closely related to *B. megaterium* and were predominant in Samples 10, 11, and 12, which were obtained after the Kosa event had finished. Several minor clones were related to *Staphylococcus hominis* and increased in Sample 6, which was obtained during the Kosa event.

Clones from *Proteobacteria* comprised 13.3% of the total clones (Table 1) and were mainly clustered in the genus *Sphingomonas* and the *Alphaproteobacteria* SAR clade (Fig. 5). The clones affiliated with *Sphingomonas* spp. were detected in almost all samples, and their numbers significantly increased at the end phase of the Kosa event (Sample 9; Fig. 2). Some clones showed 99.8% similarity with some species of *Sphingomonas* found in the northern sea, whereas other clones were closely related to *Sphingomonas paucimobilis*. Clones from the *Alphaproteobacteria* SAR clade, which contains species unique to ocean environments at the Sea of Japan and Pacific Ocean had low similarities (<97.1%) with other members of the SAR clade, suggesting that these clones represent novel bacterial species in the SAR clade. These sequences appeared randomly throughout the Kosa event as <10% of the total clones in each sample (Samples 5 and 6) and significantly increased to 50% of the total after the Kosa event finished (Sample 8).

## 4. Discussion

### 4.1. Environmental conditions and aerosol dynamics

Kosa carries bioaerosols such as bacteria, fungi, viruses, and mineral particles. Bioaerosols play an important role in microbial dispersal and have a significant impact on ecosystems, human health, agricultural productivity and climate changes in downwind areas (Jaenicke, 2005, Brown and Hovmøller, 2002). Outbreaks of Kosa over East Asian region are very frequent in the spring and last for a few days each time. In this study, the lidar measurements at Toyama city revealed that dust particles were transported to Hokuriku area (Kanazawa city) and that a Kosa event occurred between May 1 and May 4, 2011. Epifluorescence microscopy demonstrated that the several types of particles such as mineral particles (white particles), organic particles (yellow particles), and microbial particles were present in air samples collected at an altitude of 10 m. DAPI-stained particles with yellow fluorescence have been reported to resemble organic materials originating from proteins and other microbial cell components (Mostajir et al., 1995) and were observed to be present at the altitude of 3000 m over Suzu city, Japan during a Kosa event (Maki et al., 2013).

The concentration of aerosol particles increased 10– to 100–fold during the dust event (Fig. 1). Kosa events have been reported to increase the biomass of airborne microorganisms in correspondence with the amount of mineral particles (Hara et al., 2012) and significantly change bacterial species structures in the free troposphere (Maki et al., 2013). The majority of phylotypes recovered from the 12 samples belonged to the phyla *Cyanobacteria, Alphaproteobacteria,* and *Firmicutes,* and the bacterial

compositions consisting of the members from the three phyla showed significant dynamics from the initiation of the Kosa to the days after the event had passed (Table 1, Fig. 2).

### 4.2. *Cyanobacteria*

Phylogenetic analysis showed that all the clones from *Cyanobacteria* formed two clusters in the marine genus *Synechococcus* and were closely related to the *Synechococcus* spp. found in the Sea of Japan and East China Sea (Choi and Noh, 2009) and coastal areas (Fuller et al., 2013; Fig. 3). Kosa particles reaching Japan are mixed with seawater compounds when passing over the Sea of Japan (Zhang et al., 2006). *Cyanobacteria* including *Synechococcus* spp., are known to be resistant to UV irradiation and oxygenic stress because they have to eliminate excess peroxide generated from photosynthesis (Latifi et al., 2009, Perelman et al., 2003). In a previous study, marine microorganisms such as cyanobacteria were shown to be transported by a Kosa event and comprised 20% of clone libraries obtained from air samples collected from Europe regions (Polymenakou at al. 2008). Clones affiliated with *Synechococcus* spp. dominated Samples 1 and 2, which had been collected during the first phase of the Kosa event, and then decreased to no less than 30% during the sampling period (Fig. 2). It is likely that the front air mass of the Kosa event coming from the continental area would have blown the cells of marine *Synechococcus* spp., as well as seawater, up into the air; the cells and the seawater would subsequently have fallen down upon the downwind area.

### 4.3. *Firmicutes*

Sequences related to the *Firmicutes* members *B. subtilis* and *B. pumilus* comprised most of the 16S rDNA clone libraries from Samples 4, 6, and 8 (Figs. 2 and 4), which had been taken at night during the Kosa event. These samples contained higher concentrations of aerosol particles than the other samples, in accordance with the occurrence of a Kosa event (Fig. 1). Some clones showed more than 99.7% similarity with *B. subtilis* and *B. pumilus*, which were predominant among aerosols collected at high altitudes above the Taklamakan Desert (Maki et al., 2008), from an area downwind of Kosa events (Suzu city; Maki et al., 2010), and from the mineral particles collected from snowfall at Mount Tateyama (Maki et al., 2011). Species related to *B. subtilis* were isolated from sand from the Gobi Desert (Hua et al., 2007) and are reported to dominate in the surface air of Saul city during Kosa events (Jeon et al., 2011). In addition, isolates of *Bacillus* spp. carried by a Kosa event were predominant in air samples taken during a free-tropospheric sampling carried out on a North American mountain (Smith et al., 2012). It has been shown that *Bacillus* spp. form resistant endospores to enhance their survival in the atmosphere (Nicholson et al., 2000). Presumably, at night during the Kosa events, microbial particles such as *B. subtilis* and *B. pumilus* fell from the free troposphere to near ground surface levels in Kanazawa city.

Unlike to *B. subtilis* and *B. pumilus*, the *Firmicutes* species *B. megaterium* was found in the daytime during the Kosa event and dominated after the dust event finished. *B. megaterium* has rarely been detected at high altitudes above the Taklamakan Desert and Kosa arrival areas (Suzu city and Mt. Tateyama; Hau et al., 2007, Kakikawa et al., 2008). Conceivably, in the daytime, the upward flow of air caused by sunlight may

contaminate the surface atmosphere with the local bacterial population from ground surface. The trajectories indicated that after the Kosa event finished, the air mass mainly remained around Japan for a few days. Thus, it is possible that the *B. megaterium* detected in the samples originated from the local ground surface and was transported to the airborne bacterial communities by upper flow.

### 4.4. *Alphaproteobacteria*

The proportion of the clone libraries representing the *Alphaproteobacteria* members of the SAR clade and the genus *Sphingomonas* fluctuated from 13% to 25% and increased at the end phase of Kosa event and after the Kosa event finished (Samples 5, 6, and 9; Figs. 3 and 6). Members of the SAR clade (Giovannoni and Stingl, 2005) and *Sphingomonas* spp. (Eguchi et al., 1996) are known to be ubiquitous marine oligotrophic ultramicrobacteria, which are thought to demonstrate improved survival at low substrate concentrations. The SAR clade is composed of ubiquitous and unculturable marine bacteria detected in the Sea of Japan (Song et al., 2009) and in ocean areas such as the Pacific Ocean (Giovannoni and Stingl 2005). Members of *Pelagibacter*, including the SAR clade, occupy approximately 25%–50% of marine bacteria in ocean areas, indicating their ability to survive in extreme environments (Morris et al., 2002). *Sphingomonas* spp. have also been identified as being an important part of marine bacterial plankton and are often found in marine bacterial communities from north Asian areas (Dieser et al., 2010) and the polar regions (Gloeckner et al., 2000). *Sphingomonas* spp. are often found to comprise the dominant bacterial population in the free troposphere over Noto Peninsula, Japan (Maki et al.,

2013) and in cloud water (Amato et al., 2007). *Sphingomonas* spp. have been reported to be particularly resistant to elevated concentrations of oxidants such as hydrogen peroxide, one of the major sources of free radicals in cloud water, and to have the capacity to rapidly adapt to changing nutritive conditions (Eguchi et al., 1996; Ostrowski et al., 2001). Furthermore, some strains of *Sphingomonas* spp. have been repeatedly isolated from extreme and cold environments such as Arctic and Antarctic soils (Baraniecki et al., 2002) and a Greenland ice core (Miteva et al., 2004). Bacterial populations that are resistant to various environmental stressors in extreme environments, such as oceans or cold regions, would be able to extend their habitats efficiently via atmospheric transport.

### 4.5. Influences of bacterial communities on ecosystems and human societies

Clone libraries recovered from air samples from May 1 to May 7, 2011 were dominated by several bacterial species from the phyla *Firmicutes* and *Alphaproteobacteria*, which are often associated with effects on plant and animal growth and human health (Table 1, Fig. 2). The *Firmicutes* clones had high similarities with *B. subtilis*, *B. pumilus*, *B. megaterium* and *Staphylococcus* spp. Although the overwhelming majority of *Firmicutes* members are nonpathogenic bacteria, some species are well known as pathogens of plants, animals, and humans (e.g., *B. pumilus*, *Staphylococcus* spp.; Thomas and Whitte, 1991, Yoshida et al., 2001). The *Alphaproteobacteria* clones that were predominant in air samples collected after the Kosa event were related to *Sphingomonas* spp., which are reported to cause human infections (Ammendolia at al., 2004) and to be opportunistic pathogens in clinical

environments (Kilic et al., 2007). In contrast, many *Bacillus* strains have been exploited for biotechnological applications (Hou et al., 2005) and have been shown to contribute to the promotion of plant growth (Yadav et al., 2011). Thus, the transport of airborne bacteria provides a mechanism for the dispersal of bacterial populations that can have both beneficial and negative influences on human health and plant growth.

Members of the phyla *Cyannobacteria*, *Firmicutes,* and *Proteobacteria* have often been found to contribute to geochemical processes and organic-matter cycles. In natural environments, the main roles of the genus *Bacillus* in *Firmicutes* appear to involve carbon and nitrogen cycling (Das and Mukherjee, 2007, Ulrich et al., 2008). In fact, the *Bacillus* sequences that were predominant in the air samples were related to or identical to those of *B. subtilis* and *B. megaterium*, which mineralize organic matter, thus contributing to the carbon cycle in terrestrial environments (Das and Mukherjee, 2007, Ulrich et al., 2008). Moreover, members of the genus *Sphingomonas* found in the air samples included many strains that degrade organic matters such as xenobiotics and hydrocarbons (Baraniecki et al., 2002, Stolz, 2009). Members of the phylum *Cyanobacteria*, including *Synechococcus* spp. have photosynthetic abilities and sometimes form blooms that contribute to carbon dioxide cycles in marine ecosystems (Stewart and Falconer, 2008). The population of *Synechococcus* spp. in the atmosphere is possibly the seed population for forming blooms in aquatic environments. The *Pelagibacter* group including the SAR clade in *Alphaproteobacteria* comprises the most abundant and ubiquitous clade of heterotrophic bacteria in the ocean (Morris et al., 2002), and it has been suggested that the seasonal dynamics of these microorganisms contribute to organic carbon cycling in ocean (Morris et al., 2005). Therefore, the

dispersal of airborne bacterial communities may contribute to geochemical cycles and microbial-diversity maintenance in several environments.

## 5. Conclusion

This study demonstrated that the airborne bacterial communities in an area downwind of a Kosa event significantly varied between the time of the Kosa event and the non-Kosa event days during which the air-mass sources changed. In the initial phases of the Kosa event, the dust mainly included marine cyanobacteria and seawater components. Then, during the middle of the Kosa event, the members of the *Firmicutes* *B. subtilis* and *B. pumilus*, which are thought to have been carried by the Kosa event, increased. At the end phase of the Kosa event, the air mass over the north areas of the Sea of Japan transported the *Pelagibacter* spp. (SAR clade) and *Sphingomonas* spp. to Kanazawa city. When the air mass remained over Japan after the Kosa event had finished, *B. megaterium* thought to originate from the local bacterial population became predominant. Since the amount of airborne bacteria during the non-Kosa period was low, the bacterial biomass and species composition may be easily changed by immigrations of bacterial populations associated with dust mineral particles and marine bacterial populations during Kosa events. It is possible that the bacterial communities around human habitats exhibit significant differences in dynamics depending on the appearance of a Kosa event and the origin of the air masses participating in the event. The clones obtained from the air samples were related to several species that were found to be associated with plant and animal growth, human health, geochemical processes, and organic-matter cycles. In the future, the impact of airborne bacterial populations on

human societies and bio-ecosystems in environments downwind of Kosa events should be investigated using physiological experiments targeting bacterial cultures and genetic analysis of functional genes.

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**Figure Legends**

Fig. 1 Temporal variations of concentrations of all bacterial particles (open circles) (a), and white particles (closed circles) and yellow fluorescence particles (closed triangles) of 0.2–2.5 μm (b), 2.5–5.0 μm (c), and >5.0 μm in diameter (d) in bioaerosol samples collected at a 10-m altitude in Kanazawa city from May 1 to May 7, 2011.

Fig. 2 Change in compositions of the partial sequences of 16S rDNA clones (ca. 400 bp) obtained from bioaerosol samples collected at a 10-m altitude in Kanazawa city from May 1 to May 7, 2011.

Fig. 3 Phylogenetic tree including the partial sequences of 16S rDNA amplicons obtained from the clone libraries (Kzp series) from the bioaerosol samples collected in Kanazawa city and the known members of *Cyanobacteria*. The phylogenetic tree was calculated from a dissimilarity matrix of an approximately 1,400 bp alignment (*E. coli* numbering 92 to 1,475) using a neighbor-joining algorithm. The sample information and the accession number of each reference sequence are given in parentheses. Open circles at branch points indicate that bootstrap values obtained by neighbor-joining analysis exceeded 50% (after 1,000 resamplings).

Fig. 4 Phylogenetic tree including the partial sequences of 16S rDNA amplicons obtained from the clone libraries (Kzp series) from the bioaerosol samples collected in Kanazawa city and the known members of *Firmicutes*. The phylogenetic tree was

calculated from a dissimilarity matrix of an approximately 1,400 bp alignment (*E. coli* numbering 71 to 1,432) using a neighbor-joining algorithm. The sample information and the accession number of each reference sequence are given in parentheses. Open circles at branch points indicate that bootstrap values obtained by neighbor-joining analysis exceeded 50% (after 1,000 resamplings).

Fig. 5 Phylogenetic tree including the partial sequences of 16S rDNA amplicons obtained from the clone libraries (Kzp series) from the bioaerosol samples collected in Kanazawa city and the known members of *Alphaproteobacteria*. The phylogenetic tree was calculated from a dissimilarity matrix of an approximately 1,400 bp alignment (*E. coli* numbering 86 to 1,437) using a neighbor-joining algorithm. The sample information and the accession number of each reference sequence are given in parentheses. Open circles at branch points indicate that bootstrap values obtained by neighbor-joining analysis exceeded 50% (after 1,000 resamplings).

**Table 1. Phylogenetic affiliation of 16S rDNA gene sequences obtained from clone libraries.**

| Category | Clone No.*1 | of clones*2 | Sampling period*3 | Length (bp) | GenBank accession no. | Closest relative | Similarity (%)*4 |
|---|---|---|---|---|---|---|---|
| *Acidobacteria* | KZtp1-28 | 1 | 1 | 597 | AB900929 | bacterium Ellin6099 (AY234751) | 90.3 |
| *Actinobacteria* | KZtp12-17 | 4 | 1,2,12 | 602 | AB900930 | bacterium SCGC AAA071-N11 (JF488663) | 94.2 |
| | KZtp3-22 | 1 | 3 | 721 | AB900931 | *Actinoplanes pyriformis* (AJ277582\|) | 85.6 |
| *Cyanobacteria* | KZtp1-1 | 80 | 1,2,3,4,6,7,8,9,10,11 | 1479 | AB900932 | *Synechococcus* sp. CC9902 (CP000097) | 96.2 |
| | KZtp3-16 | 13 | 2,3,6,11 | 1360 | AB900933 | *Synechococcus* sp. CC9605 (CP000110) | 99.8 |
| | KZtp1-19 | 5 | 1,2,7 | 454 | AB900934 | bacterium WHC3-9 (JQ269283) | 91.2 |
| | KZtp10-6 | 4 | 1,7,10 | 479 | AB900935 | *Synechococcus* sp. CC9311 (CP000435) | 90.9 |
| | KZtp4-12 | 1 | 4 | 710 | AB900936 | *Halospirulina* sp. EF17(2012) (JX912466) | 95.5 |
| *Firmicutes* | KZtp5-8 | 96 | 2,4,5,7,10,11,12 | 1580 | AB900937 | *Bacillus megaterium* (DQ789400) | 100.0 |
| | KZtp6-42 | 60 | 6,8 | 1459 | AB900938 | *Bacillus* sp. 4115 ( JX566594) | 99.9 |
| | KZtp9-16 | 16 | 4,6,8,9 | 1432 | AB900939 | *Bacillus subtilis* (KC542358) | 100.0 |
| | KZtp2-5 | 7 | 1,2 | 1492 | AB900940 | [Brevibacterium] halotolerans (JX644589) | 99.8 |
| | KZtp8-30 | 4 | 8, 10 | 1460 | AB900941 | *Bacillus subtilis* (EF523474) | 99.7 |
| | KZtp6-1 | 3 | 6, 8 | 1515 | AB900942 | *Staphylococcus hominis* (FJ768458) | 99.9 |
| | KZtp5-10 | 2 | 5,7 | 613 | AB900943 | *Bacillus megaterium* (KF419129) | 100.0 |
| | KZtp7-43 | 2 | 7 | 600 | AB900944 | *Bacillus* sp. 6014 (JX566659) | 99.3 |
| | KZtp6-38 | 2 | 6 | 596 | AB900945 | *Bacillus* sp. H69 (KC466132) | 99.8 |
| | KZtp8-32 | 1 | 8 | 1462 | AB900946 | *Bacillus subtilis* (JQ435698) | 98.4 |
| | KZtp5-26 | 1 | 5 | 621 | AB900947 | *Bacillus* sp. M81 ( KC466182) | 99.8 |
| *Eukaryota* | KZtp4-9 | 11 | 1,4,9 | 710 | AB900948 | *Quercus nigra* chloroplast (HQ664601) | 99.9 |
| | KZtp2-13 | 11 | 2,3,4,5 | 694 | AB900949 | *Quercus nigra* chloroplast (HQ664601) | 99.7 |
| | KZtp7-13 | 6 | 7,8 | 567 | AB900950 | *Pinus merkusii* chloroplast (FJ899579) | 99.8 |
| | KZtp12-26 | 6 | 2,6,7,12 | 599 | AB900951 | *Pinus pinaster* chloroplast (FJ899583) | 100 |
| | KZtp3-19 | 1 | 3 | 717 | AB900952 | *Micromonas* sp. RCC299 chloroplast (FJ85 | 99.3 |
| *Proteobacteria* | KZtp5-4 | 21 | 5,7,11 | 1547 | AB900953 | *Sphingomonas paucimobilis* (KC017473) | 100 |
| | KZt-9-1 | 15 | 9 | 1424 | AB900954 | bacterium WHC5-1 (JQ269290) | 99.2 |
| | KZtp3-3 | 12 | 1,2,3,4,8,9,10 | 1549 | AB900955 | bacterium SH1-7 (JQ269250) | 91.6 |
| | KZtp7-24 | 2 | 7,11 | 1498 | AB900956 | *Sphingomonas paucimobilis* (KC017473) | 97.4 |
| | KZtp4-2 | 2 | 4 | 740 | AB900957 | *Escherichia coli* DH1 (CP001637) | 99.9 |
| | KZtp2-23 | 2 | 2,4 | 625 | AB900958 | *Alpha proteobacterium* IMCC10406 (FJ532 | 99.4 |
| | KZtp5-16 | 1 | 5 | 622 | AB900959 | *Herbaspirillum aurantiacum* (HQ830497) | 99.5 |
| | KZtp5-1 | 1 | 5 | 691 | AB900960 | *Cupriavidus metallidurans* (JQ659694) | 99.6 |
| | KZtp7-37 | 5 | 7 | 599 | AB900961 | *Bacterium* SH1-7 (JQ269250) | 90.9 |
| | KZtp6-14 | 3 | 6 | 535 | AB900962 | *Alteromonas macleodii* (CP004855) | 92.5 |
| | KZtp1-17 | 1 | 1 | 650 | AB900963 | *Acinetobacter calcoaceticus* (JX010982) | 99.6 |

***1 Clones of 16S rDNA library were named as the KZtp serie.**

***2 The numbers of the clones in 16S rDNA clone libraries.**

***3 Sampling period when the air sample was collected.**

***4 Similarity value between each isolate and the closest relative in databases.**

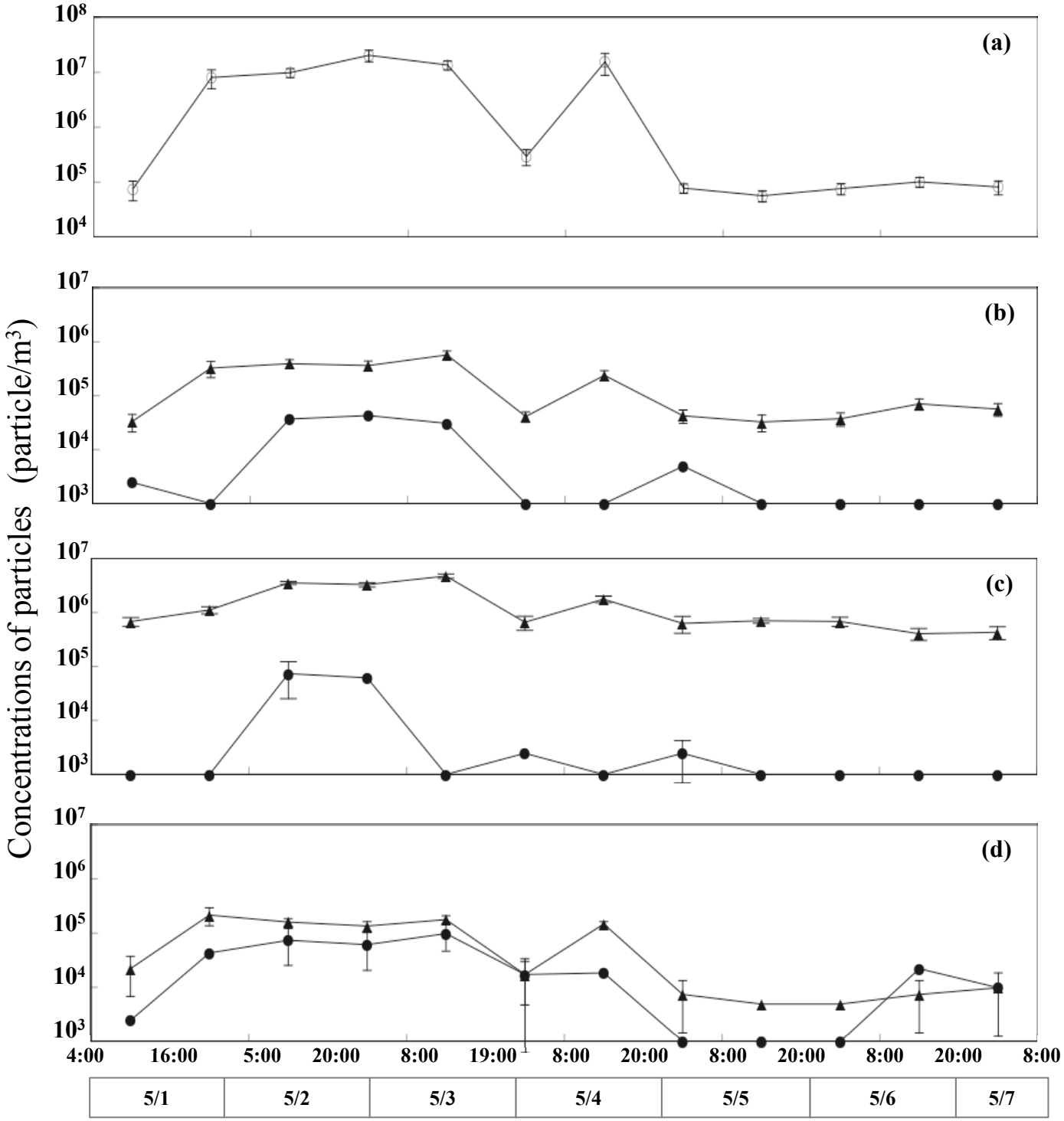


Fig.1 T.Maki et al.

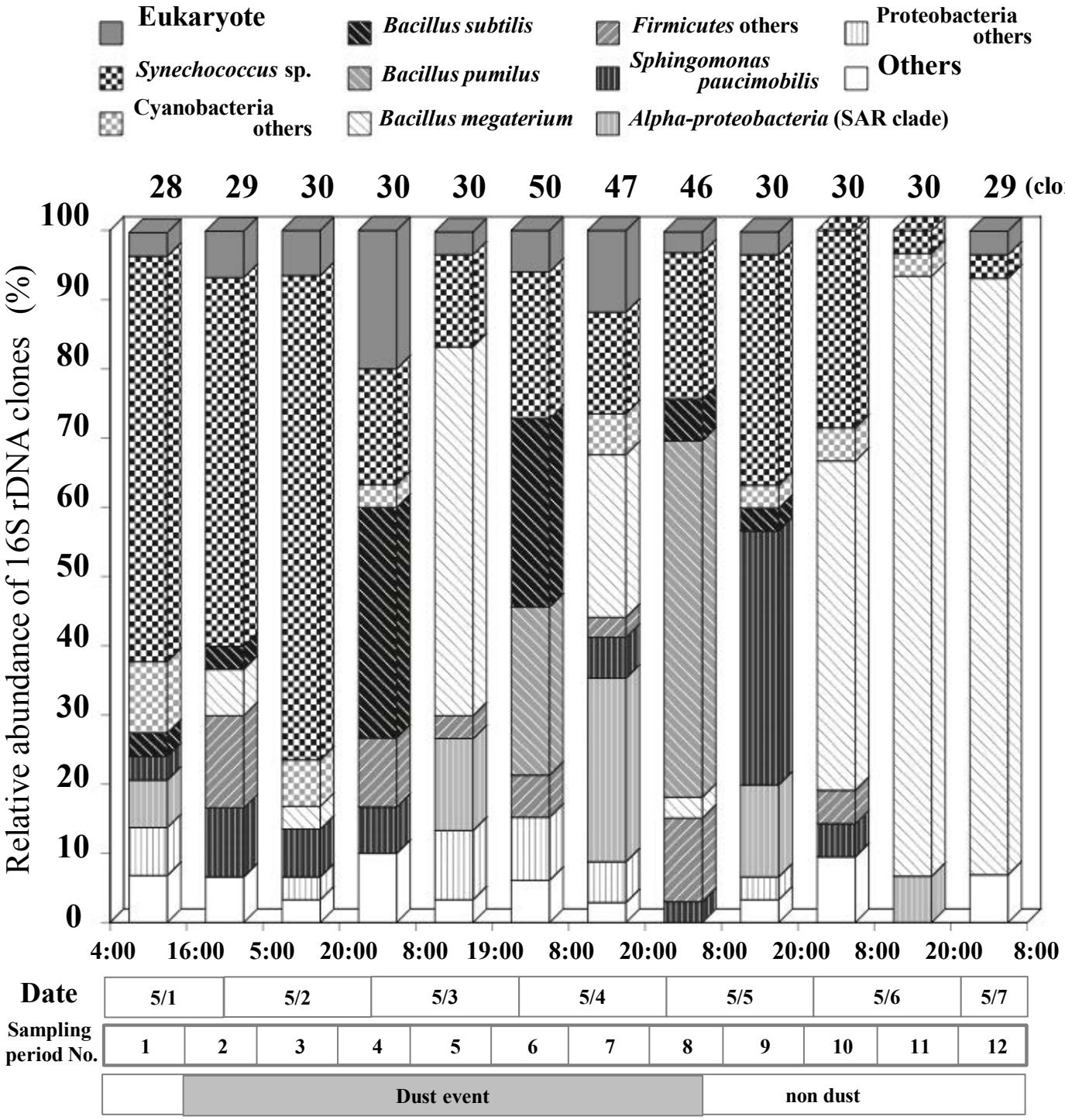


Fig. 2 T.Maki et al.

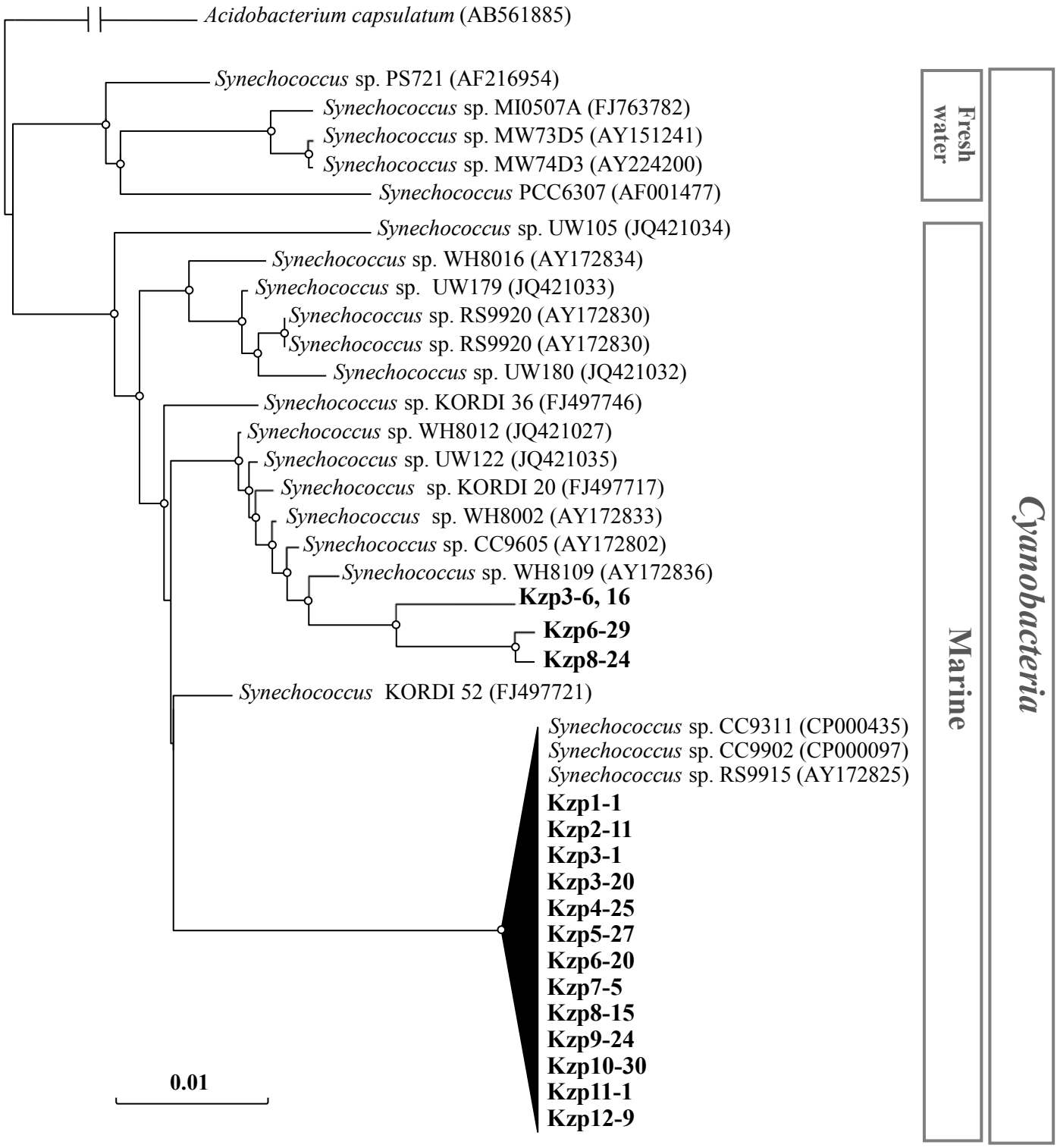


Fig. 3 T.Maki et al.

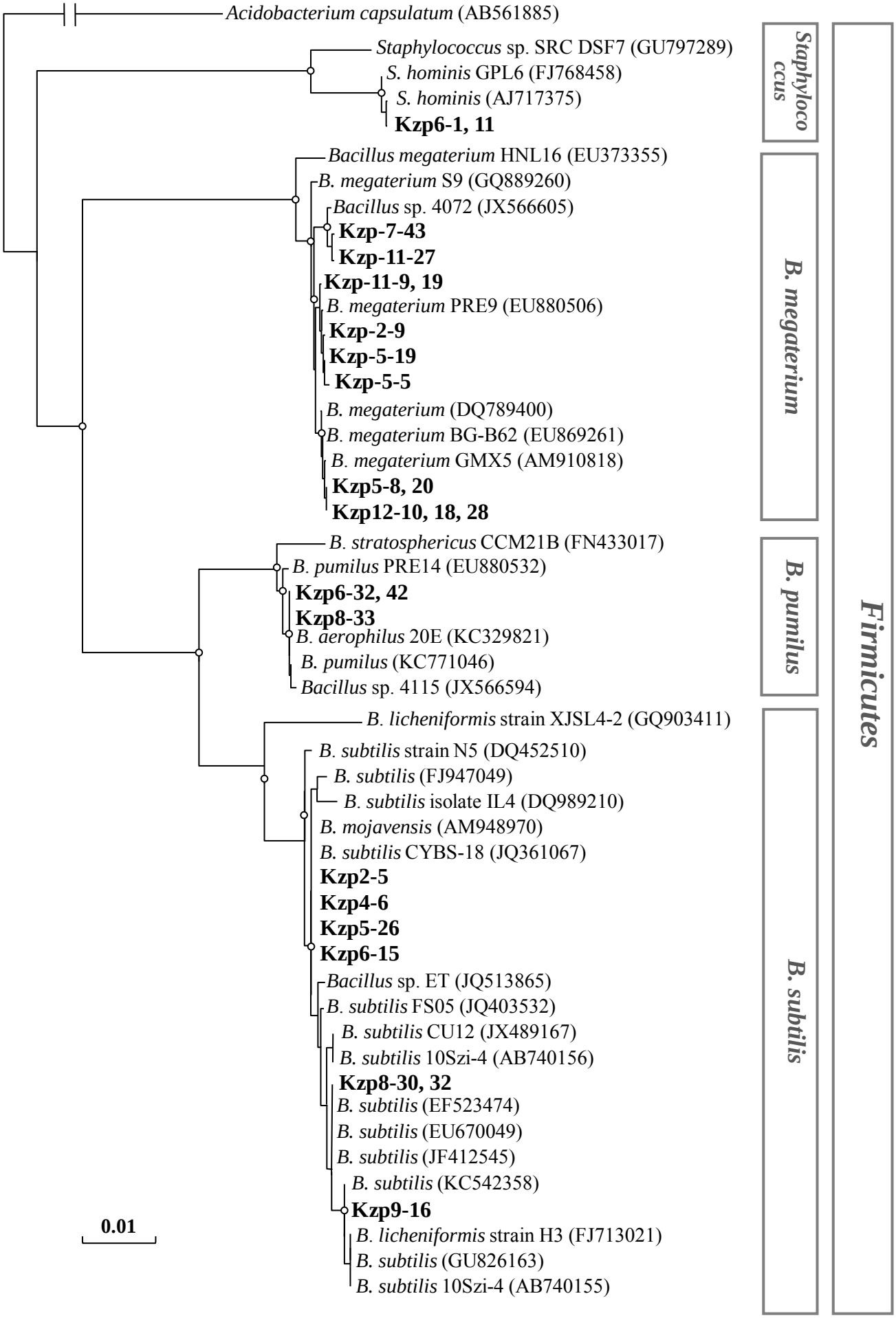


Fig. 4 T.Maki et al.

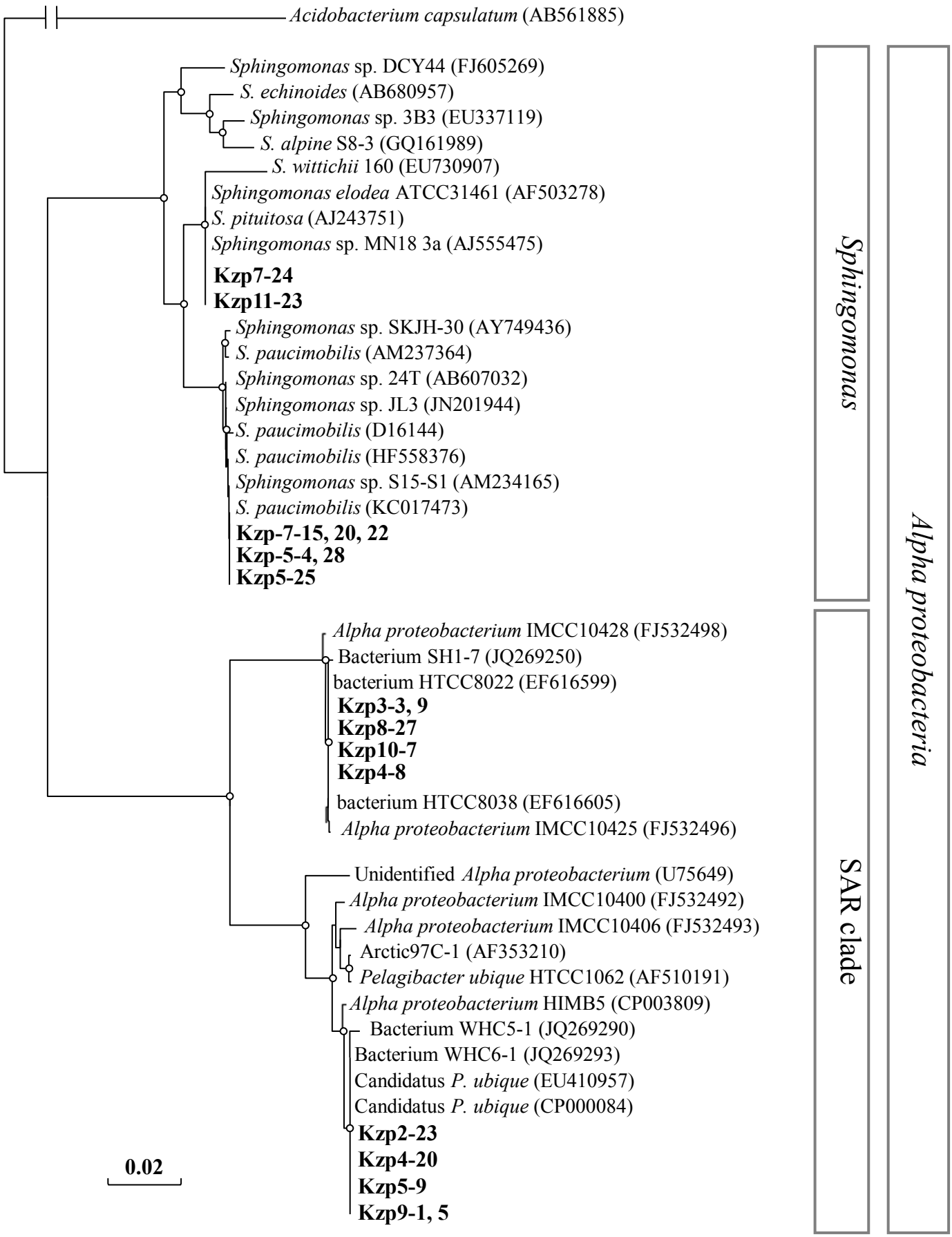


Fig. 5 T.Maki et al.