博　士　論　文

総観気象の変化に伴う大気中細菌の濃度変動

Evolution of Airborne Bacteria in Association with Synoptic Weather

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要　旨

細菌は地球上に普遍的に存在する微生物であり, その膨大な生物量と多様な代謝系は環境中物質循環において不可欠なはたらきを担っている. 大気中に浮遊する細菌は, エアロゾル粒子の一種として日射を散乱・吸収することにくわえ, 雲の氷晶核や凝結核となることで雲形成を経て大気の放射や降水に影響を与えていることが指摘されている. 細菌の大気中拡散・移動性は, 細菌の普遍的生物地理分布形成の主要な成因であるといわれ, さらに大気中での代謝活動を行うことにより大気中物質動態にも影響を与えることが予想されている. また一方で, 病原細菌の越境伝播は大気中汚染物質の長距離輸送と同様に懸念されている. これらの観点から, 大気中細菌のエアロゾル粒子としての挙動ならびに生物としての挙動について双方向から解明する必要があるが, そのための情報は非常に限られているのが現状である.

エアロゾル粒子としての細菌は空気の流れに伴って変動し, 異なる空気塊においては存在する細菌の発生源や輸送過程も全く異なると考えられる. 空気の流れは様々な要因によって生じるが, 広範囲のスケールでは総観気象の要因, すなわち, 西から東への低気圧・高気圧の移動に伴って数日単位で変化する流れが卓越する. たとえば, 春季・秋季に見られる大規模な黄砂は, 低気圧と高気圧の移動がアジア大陸の砂漠あるいは乾燥地帯の砂塵を東方に長距離輸送する流れを生じさせることによる. 本研究では, 春季・秋季の移動性低気圧および高気圧の通過に伴う大気中細菌濃度を明らかにし, 総観規模の空気塊の変化による細菌の濃度変動を定量化することを目的とした.

細菌研究は培地を用いて培養することによって行われてきた. しかし, 環境中細菌の大部分が人工環境で増殖することは困難であるといわれており, 大気中においても

実際に存在する細菌の 95%以上が培養不可能であるといわれる. 細菌の大気中挙動および雲形成への影響を解明するためにはすべての細菌の情報が不可欠であるため, 細菌 DNA や生体物質そのものを標的にした染色法や定量的 PCR 法などが有効である. 最も簡単な方法は細菌 DNA を蛍光色素で標識して計数する手法で，DAPI（4',6-diamidino-2-phenylindole）染色法が一般的である．しかし，一般的な蛍光染色法では細菌の総数は得られるが，生存状態に関する情報は得られない．そこで，本研究ではまず，細菌の総数（濃度）と生存状態の双方に対してアプローチできる方法の検証・確立を試みた.

大気中細菌の濃度および生存状態を計測するため，LIVE/DEAD BacLight Bacterial Viability Kit（BacLight 染色）を用いた蛍光染色計数法の室内・野外試験を行った. BacLight 染色では細菌 DNA の蛍光標識により細菌総数を得られるとともに，細菌膜の損傷による生細菌・死細菌の判別が可能である．大気中細菌の捕集法および前処理法の検証実験も含め，BacLight 染色法と一般的方法である DAPI 染色法との比較を行った. 大気中から分離された細菌株を用いた室内試験により, BacLight 染色法と DAPI 染色法との十分な整合性を確認した．また，1%グルタルアルデヒドによる固定処理が生細菌の検出率を向上させることを発見した．続いて，野外試験として BioSampler を用いた大気中細菌捕集を行った．BioSampler は大気中浮遊粒子を液体中に吸引・捕集する装置であり，一般的な細菌の大きさである 1 μm 前後の粒子捕集効率が高く，捕集中の細菌の損傷も比較的低減できるといわれる．これを用いて大気中細菌を 1 時間捕集し，グルタルアルデヒド固定処理を施した BacLight 染色法ならびに DAPI 染色法による結果の相互比較を行った．検証の結果として，(1) BacLight 染色法はグルタルアルデヒド固定によって DAPI 法による結果と整合性を有する, (2) BioSampler

による捕集によって 1 時間の時間分解能で大気中細菌の濃度および生存率の変動を追跡することができる，という 2 点が明らかになった．1 時間の捕集時間は気象の変化に対応可能な時間分解能であるため, 本法が空気塊の移動に伴う大気中細菌の濃度変動の解明に適応可能であることが示された.

検証した方法を用いて春季・秋季の熊本市における移動性低気圧・高気圧の通過に伴う大気中細菌濃度および生存状態の変動を観測した. また, 九州西岸域においては, 大気中粗大粒子数濃度（粒径 1 μm 以上）の数時間内の急増はアジア大陸からの空気塊の輸送によってもたらされることが知られているため, 大気中浮遊粒子数濃度は外部からの空気の流入を示す有用な代用情報となり得る．そこで，低気圧・高気圧の通過に伴う大気中浮遊粒子数と大気中細菌との相関性を調査し, 長距離輸送について検討した．低気圧に伴う寒冷前線の通過後，大気中細菌濃度と粗大粒子数濃度が同時に増加し，両者には相関がみられた．寒冷前線後面に位置する空気塊は比較的移動速度が速く, アジア大陸起源の粒子を多く含む気塊を日本あるいは北西太平洋にまで輸送することが知られる．したがって，粒子とともに増加した細菌はアジア大陸より輸送されたものが多く含まれていたと考えられる．くわえて，この濃度増加時に細菌生存率が 50%以下に低下したことから，大陸から輸送された細菌は死細菌が多く，大気中輸送時に損傷している可能性が示唆された. 高気圧時の大気中細菌と粗大粒子は比較的低濃度で相関がみられなかった．高気圧時は地表付近の空気塊があまり移動せず，停滞した空気質が形成される．このような条件下では，局地起源のエアロゾル粒子の蓄積が生じることが知られている．このことから，局地起源に由来する細菌が大気中に存在していることが示唆された．このときの細菌生存率は常に 80%以上であった．局地起源の細菌は発生源から放出されて間もないと考えられるため, 大部分が生存率

を保ったまま浮遊していたと考えられる.

春季の天草沿岸地域において同様の趣旨の観測を実施した. この地域は海岸に面しており, 熊本市よりも産業や人間活動が少なく, 人為的なエアロゾル発生源が少ない. 結果は熊本市と同様, 低気圧通過後における大気中死細菌と粗大粒子との相関と, 高気圧条件下における高い細菌生存率が確認された. 一方で熊本市の結果と異なり, 高気圧条件下の海陸風により風向が変化する時間帯の前後に生細菌濃度の急増が見られた. このとき粒子数濃度は変動せず, 周辺に細菌濃度上昇に関連するような人間活動および自然発生源がなかったことから, 海陸風によって周辺で発生した生細菌の蓄積・輸送が短時間に生じていたことが考えられる. 高気圧条件下の地表付近における空気塊の停滞は, 海陸風のような局地規模での空気塊の移動を際立たせ, それに伴う局地由来細菌の濃度変動を駆動しているといえる.

以上の結果から, 南西日本における総観気象の変化に伴う大気中細菌の濃度変動について以下のような結論を得た. 高気圧通過時の空気塊中には局地起源と考えられる生細菌が多く, 海陸風などの局地的気象要因が大気中細菌の濃度変動を駆動している. 一方で, 低気圧通過時の空気塊中には長距離輸送されたと考えられる死細菌が多く, 外部からの流入が大気中細菌濃度の変動をもたらしている. 大気中細菌と粒子との相関関係から, 外部から流入する空気塊においては細菌とエアロゾル粒子との相関が成り立つが, 停滞性の空気塊では成り立たないことが明らかとなった. 大気中細菌は自然起源の局地的発生源から一定供給がなされていると考えられるため, 停滞性空気塊においては他の粒子とは関係なく蓄積・輸送が生じたと推察される. したがって濃度変動という点に限定すれば, エアロゾル粒子としての細菌は, 遍在する局地的発生源に一定の影響を受けているという点が他のエアロゾル粒子との挙動の違いを特徴づ

けているといえる．しかし，このような粒子との挙動の違いについては，局地的発生源が大気中浮遊粒子の変動を決定づけない比較的清浄な環境においてのみ成り立つと予想される．

これまでに世界各地で観測されている地表付近大気中細菌濃度は平均的に 10^5–10^6 cells m^{-3} のオーダーであり，本研究で観測されたオーダーと一致していた．また，過去様々な調査地点において，大気中細菌濃度は 1 桁の範囲で変動することが報告されており，本研究における濃度変動範囲と一致した．本研究による濃度範囲は，低気圧による細菌の輸送と高気圧下の海陸風に伴う細菌の急増により形成されていた．本研究において，過去の多くの研究と同じ一桁の変動幅を示すということは，総観気象の変化による空気塊の移動および海陸風が大気中細菌の濃度変動をもたらす主要因の一つであることを示している．

Abstract

Airborne bacteria are the major group of bioaerosols. Data on the concentration and viability of bacteria in the atmosphere are crucial for understanding their dispersion, roles in environmental changes, and effects on ecosystems. However, the evolution and distribution of bacteria in the air are poorly understood due to the lack of accurate information at an appropriate temporal resolution.

In this study, we first investigated the applicability of the LIVE/DEAD® BacLight™ Bacterial Viability Kit (BacLight stain) with regard to the enumeration of viable and non-viable bacterial cells in the air, with the 4',6-diamidino-2-phenylindole (DAPI) stain used as the control of total cell counts. Two cultured bacterial strains isolated from an air sample, *Bacillus subtilis* and *Micrococcus* sp., were used in laboratory experiments. The results of BacLight staining agreed well with those of DAPI staining in detecting total cell counts, and the detection efficiency of *Bacillus subtilis* was 76–112%. Bacterial viability (number ratio of viable cells to total cells) showed consistency among repeated experiments with the same sample replicates, indicating the high confidence of counting viable cells, as well as total cells with the stain. Fixation of samples with glutaraldehyde prevented fluorescence bleaching at the exposure to fluorescence and increased detection accuracy. Application of the BacLight stain with and without fixation to air samples that were collected with a bio-sampler at an urban site proved the effectiveness of this approach in determining the cell concentration and viability of airborne bacteria at a 1-hour time resolution. A combination of BacLight staining and glutaraldehyde fixation treatment, in parallel with experiments without this treatment in field measurements, is proposed to ensure the detection accuracy.

Applying the method to field observations, we investigated the evolution of viable and non-viable bacteria in the air with synoptic weather changes in Kumamoto city on the

southwestern Japan coast. The purposes were to outline the manner by which bacteria were transported and to quantify the bacterial abundance and viability in the Northern Hemisphere westerly winds. The observations were conducted when the weather was governed by cyclones or anticyclones associated with Asian continent outflow between 12 October 2011 and 7 April 2013.

Bacterial concentration in thermodynamically different air parcels was in the same order but different ranges: 4.5×10^5–1.3×10^6 cells m^{-3} in postfrontal air, 2.4×10^5–1.4×10^6 cells m^{-3} in prefrontal air, 5.4×10^5–9.9×10^5 cells m^{-3} in the air between cyclones and anticyclones and 2.9×10^5–4.1×10^5 cells m^{-3} in anticyclone air. In postfrontal air, the concentration correlated closely with coarse aerosol particles (diameter > 1.0 μm). In contrast, bacteria did not show a correlation with coarse particles in prefrontal air and anticyclone air. Bacterial viability was approximately 70% on average of all samples. However, the viability in postfrontal air was smaller than 60% if cases of stationary fronts with stagnant air were excluded. These results indicate that air parcels following fast-moving cold fronts in the westerly wind flow constantly and efficiently conveyed airborne bacteria from the Asian continent toward northwestern Pacific and the bacteria were characterized by coarse particle-correlated high abundance and low viability. The bacteria in slowly moving anticyclone and prefrontal air, characterized by low abundance and high viability, were more likely a mixture of long-range transported ones and regionally or locally originated ones.

To further investigate the association of airborne bacteria with synoptic weather under less influence of anthropogenic activities, the concentration and viability of airborne bacteria at a seaside site, Amakusa, on the southwestern coast of Japan were investigated in the springs of 2012–2014. During the passage of cyclones, bacteria in postfrontal air were closely linked to aerosol particles; their concentration was 0.3–17 $\times 10^5$ cells m^{-3}, and the viability was 23–84%. In prefrontal air, the bacterial concentration was 3.9–8.3 $\times 10^5$ cells m^{-3} with the

viability of 64–76%, both of which varied over smaller ranges than those in postfrontal air, and no correlation between the bacteria and the particles was observed. When anticyclones were approaching the site and/or covering the site, the bacterial concentration was approximately 4–5 × 10^5 cells m^{-3} with up to two-fold pulsed increases at the alternative wind changes of land/sea breezes. The bacterial viability was usually more than 70% and the pulse increases were attributed to the local accumulation of viable bacteria when the wind changed to land/sea breezes. These results indicate the significant influence of land/sea breezes on bacterial concentration at the seaside area, in addition to the influence of cyclone passages.

The comparison between two sites, Kumamoto and Amakusa, showed that bacterial concentration was affected by locations as much as about 40%, while the viability did not significantly differ with locations. The result indicates that the viability likely represents whether bacteria are dominated by long-distance transported ones or not.

The comparison of available data at various geographical areas revealed that the global range of bacterial concentration in the near-surface air was on the order of 10^4–10^5 or 10^5–10^6 cells m^{-3} worldwide except in agricultural areas. The ranges of airborne bacteria found in this study were also on the order of 10^5–10^6 cells m^{-3}, and the high concentrations were prompted by synoptic and local scale airflows. These results suggest that dynamics of airborne bacterial abundance and viability governed by air parcel fluctuations is crucial to the exploration of the roles that bioaerosols play in the environment via the atmosphere.

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Chapter 1.

General Introduction

Bioaerosols are referred to as airborne particles originating from biological origins, including viruses, bacteria, fungi, pollen, plant debris and their derivatives, and constitute a fraction of atmospheric aerosols [*Hurst et al.*, 2002; *Jaenicke*, 2005]. They are released from surface water, soil, and plants by wind erosion, water splash and wave action. Their size ranges from tens of nanometers to hundreds of micrometers [*Hurst et al.*, 2002]. Recent studies demonstrate that airborne bacteria, as a major component of bioaerosols, play an important role in the Earth environment (Figure 1.1) [*Creamean et al.*, 2013; *Griffin*, 2007; *Iwasaka et al.*, 2009; *Pratt et al.*, 2009; *Vaïtilingom et al.*, 2013; *Womack et al.*, 2010]. In addition to their contribution to the bio-materials such as protein and fat in atmospheric aerosols, airborne bacteria can function as efficient nuclei for ice cloud formation and affect the hydrological processes and radiation transfer in the atmosphere, i.e. their climate effects [*Christner et al.*, 2008b; *Morris et al.*, 2011; *Sun and Ariya*, 2006]. The dispersal of bacteria following with airflows links the bacterial communities in geographically isolated regions [*Kellogg and Griffin*, 2006] and blurs the distinction of closely related species in distant areas [*Fenchel and Finlay*, 2004].

In order to elucidate the diversity and geography of bacteria in the atmosphere and their environmental effects, one needs to know their concentration and viable status in space and time under different weather conditions. For example, the cell number concentration of total bacteria is crucial to ice cloud formation because both live and dead cells and even cell fragments can act as nuclei for icing [*Christner et al.*, 2008a; *Möhler et al.*, 2007]. Bacterial viability is primary information to assess the ecological

effects because settling of live bacteria to the surface potentially alter the indigenous microbial composition of the deposition regions. Unfortunately, very little is known about the total cell concentration and the viability of airborne bacteria in the atmosphere because of the lack of appropriate approaches. Describing the cell concentrations of live and dead bacteria and their variation in space and time is still a great challenge toward understanding their impacts on atmospheric physics and chemistry and on the development and evolution of ecosystems.

Bacteria move from one place to another through the air by airflows, which, in principle, obey the rules of fluid dynamics. Bacteria can settle onto surfaces via dry and wet deposition [*Monteil et al.*, 2014], although few data are available on bacterial movement with/in airflows and on how their concentration and viability correspond to weather changes, which leaves very large uncertainties in studies to reconstruct and understand bacterial dynamics with numerical model simulation and field observation.

Progresses in the field of meteorology have enabled the accurate prediction of airflow on a synoptic scale over several days. For example, backward trajectory analysis, such as the NOAA HYSPLIT model, can calculate the trajectory of air parcels and estimate airflow over several thousands of kilometers [*Draxler and Hess*, 1998], from which we can recognize that mid-latitude cyclones in synoptic weather systems drive the long-distance movement of air parcels. The air associated with cyclones usually consists of two thermodynamically distinct sectors: prefrontal air and postfrontal air. The prefrontal air is a warm and humid air sector ascending to the upper troposphere before a front [*Carlson*, 1980]. The postfrontal air is a cold and dry air sector descending from the upper troposphere after a front [*Browning*, 1997; *Carlson*, 1980]. These airflows are the major pathways for the trans-boundary

transport of pollutants from the Asian continent toward the northwestern Pacific [*Heald et al.*, 2006; *Kaneyasu et al.*, 2000; *Liang et al.*, 2004; *Liu et al.*, 2003]. In contrast to cyclones, anticyclones cause the subsidence of air masses from the upper troposphere and trap them in anticyclone areas. The movement of air in anticyclones is stagnant, which is favorable to the accumulation of airborne particles emitted from local sources as well as those transported over long distances [*Thishan Dharshana et al.*, 2010; *Wei et al.*, 2011].

The concentration of bacteria in the air has generally been reported about the range of 10^4–10^6 cells m^{-3} on average [*Bowers et al.*, 2012; *Bowers et al.*, 2011a; *Bowers et al.*, 2011b; *Chi and Li*, 2007; *Cho and Hwang*, 2011; *Hara and Zhang*, 2012; *Harrison et al.*, 2005; *Maki et al.*, 2013; *Tong*, 1999; *Tong and Lighthart*, 2000; *Xia et al.*, 2012]. In terrestrial sites, the concentration is usually reported about 10^5–10^6 cells m^{-3} [*Bowers et al.*, 2011a; *Bowers et al.*, 2011b]. As for remote areas such as maritime sites and high alpine sites, the concentration is about two orders of magnitude lower, which is usually less than 10^4–10^5 cells m^{-3} [*Aller et al.*, 2005; *Bowers et al.*, 2012; *Griffin et al.*, 2001]. Recently, desert dust storms have been recognized as an efficient means of inputting bacteria into the atmosphere, and the long-distance movement of dust plumes could bring a substantial amount of bacteria to the places where the plumes pass. Remarkable increases in the concentrations of bacteria associated with dust particles have been observed in East Asia and Africa [*Griffin et al.*, 2001; *Hara and Zhang*, 2012; *Jeon et al.*, 2011; *Maki et al.*, 2013; *Prospero et al.*, 2005], and the amplitude of the increases ranges between several times and orders of magnitude compared to non-dust conditions [*Hara and Zhang*, 2012]. The trans-Pacific transportation of biological particles with dust from the Asian continent has even been confirmed even in the Western United States [*Creamean et al.*, 2013].

Recently, efforts have been made to uncover the dependence of the concentration and viability of bacteria on thermodynamically different air parcels. Using DNA-based methods coupled with gas and aerosol measurements and backward trajectory analysis, [*Smith et al.*, 2012; 2013] demonstrated that westerly winds conveyed bacterial cells from Asia to North America. That means the concentrations of bacteria and their variation in the air should be influenced by long-distance transport via air parcels as well as indigenous emission, and the dominance of local and/or transported bacteria should depend on the airflow under synoptic weather.

From these points, the ultimate goal of this study was set to elucidate the dynamics of airborne bacteria in association with synoptic weather. The applicability of BacLight staining method was first carefully investigated by lab- and field-tests for the measurement of airborne bacterial concentration and viability at an appropriate time resolution for weather changes (Chapter 2). Then the method was applied to quantify the airborne bacteria in Kumamoto and Amakusa in southwestern Japan, when cyclones and anticyclones approached and passed the sites (Chapter 3 and Chapter 4). In Chapter 5, the general discussion is made to figure out the dynamics of airborne bacteria with the change of synoptic weather.

Figure

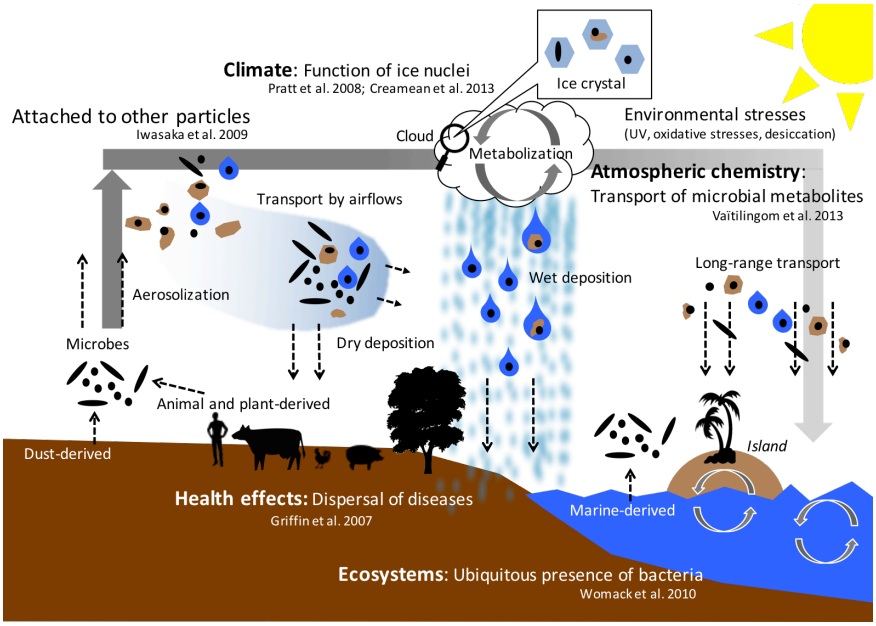

Figure 1.1. Possible roles that bioaerosols play in the environment via the atmosphere.

Chapter 2.

Applicability of LIVE/DEAD BacLight Stain with Glutaraldehyde Fixation to the Measurement of Bacterial Cell Concentration and Viability in the Air

2.1. Introduction

Airborne bacteria are usually detected with culture-based approaches. Their abundance is estimated by counting bacterial colonies forming on a nutrient agar plate after incubation for hours or days [*Hurst et al.*, 2002]. This method is an easy and convenient approach and suitable for routine measurements because of its low cost and high time resolution. The disadvantage of this approach is that only culturable bacteria can be detected and investigated with the colony-forming unit on the culture media rather than the abundance of bacteria. It is well known that only a small percentage of bacteria in a natural environment (usually less than 1–10%) can grow on selected nutrient media, and most bacteria are "viable but non-culturable" or dead [*Amann et al.*, 1995; *Lighthart*, 2000; *Maier et al.*, 2000; *Roszak and Colwell*, 1987].

A number of culture-independent approaches have been developed to pursue the abundance of bacteria in the air. The metagenomic technique is a powerful one, which is being widely used to identify airborne bacteria [*Kakikawa et al.*, 2008; *Maki et al.*, 2010; *Maki et al.*, 2008; *Nishimura et al.*, 2010]. Genes of bacteria are excellent candidates for phylogenetic analysis and a small subunit of rRNA of bacteria can offer sufficient information on the bacterial composition. Application of quantitative polymerase chain reaction (qPCR) allows access to quantitative information of target genes or target organism in air samples [*Peccia and Hernandez*, 2006]. Recently, EMA-qPCR (ethidium monoazide qPCR) was introduced to measure viable bacteria

in samples by eliminating DNA of dead cells from the analysis [*Georgakopoulos et al.*, 2009; *Rudi et al.*, 2005]. These metagenomic techniques are very efficient approaches to identify bacteria in samples but not good at the quantification of bacteria. They identify the integral abundance of bacteria in DNA amounts rather than cell counts [*Després et al.*, 2012; *Georgakopoulos et al.*, 2009; *Peccia and Hernandez*, 2006]. Bacterial number concentration can only be obtained via a transfer from DNA amounts to cell number by assuming a relation between DNA amounts and cell number [*Kakikawa et al.*, 2008].

Enumeration with fluorescent staining is a traditional technique to detect the abundance of bacteria in the air. So far the most frequently used fluorescent dye for bacterial enumeration in air samples is 4',6-diamidino-2-phenylindole (DAPI). This dye binds the DNA of bacterial cells and makes microbes appear blue under fluorescence. It has been used to measure the abundance of airborne bacteria in various environments, such as indoor air [*Li and Huang*, 2006], outdoor air [*Chi and Li*, 2007; *Li et al.*, 2011; *Rodríguez de Evgrafov et al.*, 2010], and high elevation sites [*Bowers et al.*, 2009; *Bowers et al.*, 2012; *Xia et al.*, 2012]. The cell concentration of bacteria can be obtained with this stain but the viable status of the bacteria is not available because it dyes all bacterial cells the same color. *Chi and Li* [2007] measured the viability of airborne bacteria (the number ratio of viable bacterial cells to total bacterial cells) by measuring total and non-viable cells with different dyes and obtained the viability by subtracting non-viable cell counts from total cell counts. A counter, the Ultraviolet Aerodynamic Particle Sizer (UV-APS) which is a fluorescence-based instrument, has been developed to count the real-time number of live bioaerosol particles in the air. Its results include not only live bacteria but also other bioaerosol particles, and it cannot measure the total concentration including live

and dead ones in parallel [*Brosseau et al.*, 2000; *Després et al.*, 2012; *Hairston et al.*, 1997; *Huffman et al.*, 2010; *Xu et al.*, 2011]. Recently, *Park et al.* [2012] developed a method based on ultraviolet and visible spectroscopy and succeeded in measuring rapidly the viability of bioaerosols in laboratory-prepared samples.

Despite the development of new powerful methods, there are few data available for the assessment of the effects of airborne bacteria on the environment. Primary modeling studies suggested bioaerosols were of minor importance to global ice nuclei concentrations and precipitation processes [*Hoose et al.*, 2010a]. However, recent field studies of cloud and precipitation in the western United States showed the substantial role of dust and bioaerosols in the mid-level ice cloud formation where precipitation processes were initiated [*Creamean et al.*, 2013]. A large discrepancy between modeling and observation remains because of the lack of observational data that allow proper parameterization of bacteria-associated processes in model simulations. Therefore, an accessible method is needed to easily gather the data of bacterial abundance and viability in the air. Developing a reliable method would permit interdisciplinary collaboration for wide observation campaigns of airborne bacteria as well as to provide reference data for a verification of traditional methods or other new technologies.

Hara and Zhang [2012] challenged measuring the concentration of viable and non-viable bacterial cells in atmospheric dust samples with the LIVE/DEAD® *Bac*Light™ Bacterial Viability Kit and found the effectiveness of the kit at a high sampling-time resolution by coupling with an efficient sampler. The fluorescent staining dye, LIVE/DEAD® *Bac*Light™ Bacterial Viability Kit (Invitrogen™, Molecular Probes Inc.) was developed to assay the viability of bacteria. This stain is able to distinguish between live and dead bacteria by dying them different colors.

Although there is still argument on how to define dead bacteria [*Hannig et al.*, 2010], this stain can offer the viability of bacteria as well as the bacterial abundance and has been used to study bacteria in water [*Boulos et al.*, 1999], soil [*Janssen et al.*, 2002], cloud water, and precipitation [*Bauer et al.*, 2002].

In this study, the applicability of the BacLight stain to enumerate viable and non-viable bacteria in the air, where bacterial concentration is remarkably lower than in water and soil, was carefully examined. The improvement with fixation treatment of samples was furthered with laboratory experiments. The application to atmospheric bioaerosol samples was tested. A method suitable for the detection of the abundance and viability of bacteria in the air is offered.

2.2. Method

2.2.1. Laboratory test

Bacterial samples

Two cultured bacterial strains, *Bacillus subtilis* (hereafter described as *B*. *subtilis*; accession number JN092588 from DDBJ/EMBL/GenBank databases) and *Micrococcus* sp. (accession number GU073283), were adopted in laboratory experiments. Both strains were isolated from the culture of a sample collected for airborne bacteria detection at 1000 m altitude over the sea area close to Goto Island (126.9ºE, 31.3ºN), Nagasaki, Japan during an aircraft mission on 11 December 2010. *B*. *subtilis* and *Micrococcus* sp. are both gram-positive bacteria and mainly inhabit soil [*Madigan et al.*, 2012]. *Bacilli* are spore-forming bacteria that are more resistant against environmental stress than other nonspore-forming ones [*Roszak and Colwell*, 1987]. Similar bacterial strains are frequently present as the dominant species among culturable bacterial fraction in the atmosphere [*Fang et al.*, 2007] and have been well

detected in surface and elevated air in East Asia with cultural method and DNA analysis [*Fang et al.*, 2007; *Harrison et al.*, 2005; *Hua et al.*, 2007; *Kobayashi et al.*, 2011; *Lee et al.*, 2009; *Maki et al.*, 2010; *Maki et al.*, 2008; *Smith et al.*, 2010; *Womack et al.*, 2010; *Yukimura et al.*, 2009].

For each experiment of bacterial fluorescent staining and enumeration, a set of *B. subtilis* or *Micrococcus* sp. replicates was prepared from one strain. To prepare one set of samples, a strain of *B. subtilis* or *Micrococcus* sp. was inoculated into 3 mL of Bacto™ Tryptic Soy Broth (Becton Dickinson and Company) and incubated at 30 ºC for 12–20 hours in a shaking incubator at 125 rev min^{-1} (OD595 ≈ 2.0). Then 100 µL of incubated vegetative cells were transferred into 10 mL of 0.9% saline (pre-filtered through 0.2 µm pore filters), which was further separated into several parts for subsequent staining.

Fluorescent staining

The nucleic acid binding fluorescent dye, LIVE/DEAD®*Bac*Light™ Bacterial Viability Kit L13152 (hereafter BacLight stain; Invitrogen™, Molecular Probes Inc.) was used to stain samples. BacLight stain is composed of two dyes: SYTO 9 and propidium iodide (PI). SYTO 9 penetrates all bacterial membranes (intact and injured) and labels bacterial cells green. PI can only penetrate injured bacterial membranes and labels the bacterial cells red while diminishing the green stained by SYTO 9. We consider bacterial cells with injured membranes to be non-viable (dead) cells and those uninjured to be viable (live) cells following the development of this stain. Thus, in this study, bacterial cells green under fluorescence were identified as viable ones and those red as non-viable ones. The concentration of SYTO 9 in the BacLight stain solution was 6 µM and that of PI was 30 µM. The amount of the dye solution applied

to each sample for staining was 1/100 of the sample volume.

For comparison and quality control, the traditional dye 4',6-diamidino-2-phenylindole (DAPI; Dojindo Molecular Technologies Inc.) was used to stain bacterial cells in samples for enumeration. DAPI binds AT-rich region of nucleic acid and stains all bacteria blue under fluorescence. The concentration of DAPI in the stock solution was 50 μg mL^{-1} and the dye solution applied to each sample was 1/100 of the sample volume.

The staining was conducted under complete dark conditions at 4 °C. Staining time for each sample was 15 minutes. After the staining of a sample, it was filtered through a 0.2 μm-pore-size black polycarbonate membrane filter (ADVANTEC®, Toyo Roshi Kaisha Ltd.). Then, the filter was placed on a glass slide. 10 μL of immersion oil (TypeFF, Cargille-Sacher Laboratories, Inc.) was dropped before it was covered with a cover slide.

Observation and enumeration of bacteria

Stained samples were viewed and photographed by using an epifluorescence microscope (Eclipse 80i, Nikon Corp.) equipped with a 100 W mercury lamp. Filters of 450–490 nm excitation and 520 nm emission were used for the enumeration of BacLight-stained samples and filters of 360–370 nm excitation and 400 nm emission were used for the enumeration of DAPI-stained samples.

The experiments were carried out in 3 rounds. One BacLight-stained sample and one DAPI-stained sample of *B. subitilis* were subjected to the enumeration in each experimental round. For each sample, 3 pictures were taken in different fields under the microscope. Bacterial cells were counted from 3 random fields (area of each field: 30 μm × 30 μm = 900 μm^2) in each picture and bacterial abundance in the sample was

estimated from the 9 fields.

Fluorescence bleaching and fixation with glutarladehyde

The color of stained samples actually faded gradually after exposure to fluorescence under the microscope, resulting in the fluorescence bleaching of stained bacteria. Pictures of stained bacteria had to be taken quickly. Delay of picture taking or an extension of exposure of a stained sample to fluorescence would cause a large uncertainty in its enumeration. We found that color fading still happened even though we used the neutral density filters of the microscope to reduce the fluorescence irradiation to samples and made an effort to photograph samples within 30 seconds of exposure.

In order to test if the fading could be minimized with fixation agents, some samples of *B. subtilis* and *Micrococcus* sp. were treated with glutaraldehyde (25% solution, Wako Pure Chemical Industries, Ltd.) before staining. Glutaraldehyde is a 5-carbon dialdehyde that displays potent bactericidal, fungicidal, mycobactericidal, sporicidal, and virucidal activity [*Russell*, 1994]. It can strongly fix microorganisms or other biological cells by linking amino groups in the cell membranes and is widely used as a fixation agent in both laboratory studies and field observations [*Gorman et al.*, 1980; *Kepner and Pratt*, 1994; *Tietjen and Wetzel*, 2003]. In this study, the fixation was conducted with 1% (v/v) glutaraldehyde in dark at 4 °C for 30 minutes according to previous studies [*Kepner and Pratt*, 1994].

Fixed samples, and also samples without the fixation treatment, were stained with the BacLight stain or the DAPI stain and were subjected to the subsequent epifluorescence microscopic observation. *B. subtilis* samples were also subjected to enumeration as described above. The experiments of staining the fixed and unfixed

samples with Baclight stain and DAPI stain were carried out in three rounds. To investigate the fluorescence bleaching due to the exposure and the improvement by the fixation, results from fixed and unfixed samples at the beginning of exposure and after 1-minute exposure were compared. This is because 1 minute is the common required time limitation of stained bacteria exposure to the fluorescence in microscopic enumerating. A flow chart summarizing the laboratory test was depicted in Figure S1.

2.2.2. Field test

The staining coupled with and without glutaraldehyde fixation treatment was tested to quantify bacteria in the open air. Six samples were collected on a balcony of a building in the Prefectural University of Kumamoto, Japan (32º48'N, 130º45'E; 20 m above the ground; Figure S2) between 8 and 26 January 2012. A BioSampler® (SKC Inc.) which combined impingement into a liquid with centrifugal motion was used to collect airborne bacterial samples (Figure S3). The sampler with the liquid impingement can recover culturable cells of bacteria and fungi as much as 10× greater than usual membrane filtration methods [*Griffin et al.*, 2011]. The Biosampler is more efficient than impingers in collecting air-borne bacteria because it suppresses bacterial re-aerosolization by swirling the collection liquid medium. Its collection efficiency for particles of 0.3, 0.5, 1.0, and 2.0 μm were 79, 89, 96 and 100%, respectively [*Fabian et al.*, 2005; *Willeke et al.*, 1998]. The inside of the BioSampler was coated with dimethyl polysiloxane (L-25, Fuji systems Corp.) in order to minimize the adherence of microbial cells onto the glass surface. Before sample collection, the BioSampler was washed with particle-free water (pre-filtered through a 0.025 μm pore-size filter) and heat-sterilized at 180 ºC for 2 hours.

For the collection of each sample, 20 mL particle-free phosphate buffered saline (PBS, pH = 7.4) that had been autoclaved at 121 ºC for 20 minutes was used to collect airborne bacteria. PBS could prevent possible damage of bacterial cells due to osmotic pressure change during sample collection. The sampling flow rate was 12.5 ± 0.6 L min^{-1} and the sampling time was 60 minutes. To ensure the collection efficiency, the PBS was checked and loss was compensated every 20 minutes. The look of the collection is shown in Figure S4. After the sampling, the PBS was transferred to a centrifuge tube and adjusted to 25 mL with particle-free water. Then the sample was separated into three equal portions. Two were treated with the glutaraldehyde fixation and then stained by the BacLight stain and the DAPI stain, respectively. One was directly stained by the BacLight stain. Blank samples were prepared and enumerated with the same procedures in three experiments, except that the PBS was not used for sampling. Bacterial counts in the blank samples were found 1–2 orders smaller than in the air samples.

In previous tests with airborne bacteria samples, we found that many bacteria were hardly identified with microscope photographs because of their small size. For this reason, bacteria in the samples of this study were counted directly from the microscope fields. Bacterial counting was conducted in 20 random 100 μm × 100 μm fields for each sample. The bacterial concentration (C) in the air was calculated with the counts from the 20 fields, according to

$$C_{\text{total}} = \frac{(N_{\text{viable}} + N_{\text{non-viable}}) \times S \times V_{\text{media}}}{S_{\text{field}} \times V_{\text{filtered}} \times V_{\text{air}}} \quad (1)$$

$$C_{\text{viable}} = \frac{N_{\text{viable}} \times S \times V_{\text{media}}}{S_{\text{field}} \times V_{\text{filtered}} \times V_{\text{air}}} \quad (2)$$

$$C_{\text{non-viable}} = \frac{N_{\text{non-viable}} \times S \times V_{\text{media}}}{S_{\text{field}} \times V_{\text{filtered}} \times V_{\text{air}}} \quad (3)$$

where N is the number of bacterial cells in each field, S is the area of the filter, V_{media} is the volume of PBS, S_{field} is the area of each microscopic field, V_{filtered} is the volume of filtered PBS, and V_{air} is the volume of sample air. Counting viable bacteria in each field was accomplished as soon as possible (all less than 30 seconds), in an effort to minimize the probable uncertainties due to bacterial bleaching. A flow chart summarizing the laboratory test was depicted in Figure S5.

2.3. Results

2.3.1. Laboratory test

In this section, we first compare the results of *B. subtilis* and *Micrococcus* sp. samples stained with BacLight stain and DAPI stain. Then we report the influence of the exposure of stained samples to fluorescence on the color of bacterial cells. Finally, results of the application of the fixation agent compared with those without fixation are described.

BacLight staining and comparison with DAPI staining

Figure 2.1 illustrates fluorescence microscopy photographs of *B. subtilis* and *Micrococcus* sp. samples after the BacLight staining and the DAPI staining. There were a number of green cells in addition to red ones in the BacLight staining pictures, indicating the presence of viable bacterial cells. In the DAPI staining pictures, all bacterial cells were blue. DAPI staining is unable to distinguish viable and non-viable cells, and the results from this staining are the total cell counts.

Figure 2.2 shows the statistics of bacterial cell detection of *B. subtilis* samples.

Micrococcus sp. samples were not available for cell counting because they were too aggregated to enumerate. Some cells of *B. subtilis* were also aggregated on the filter. Bacterial cells that could be identified by their rod shape in the aggregates were enumerated. But if there were cells overlapped by others, they could not be seen and counted under the microscope. In the first round of experiments, there were about 50 (37 viable and 13 non-viable cells on average) *B. subtilis* cells in one field of the BacLight-stained sample and there were about 66 cells in one field of the DAPI-stained sample. The figures were 47 (33 viable and 14 non-viable cells) and 42 in the second round of experiments, and were 49 (30 viable and 19 non-viable cells) and 61 in the third round of experiments. The detection efficiency of the BacLight staining was 76–112% in comparison with the DAPI staining, suggesting that BacLight stain is adequately efficient in the detection of *B. subtilis*. Primary laboratory investigations by Hara and Zhang (2012) showed a detection efficiency of 104%. Therefore, BacLight stain is a suitable dye for detecting the abundance of bacteria such as *B. subtilis* and it can distinguish viable and non-viable cells besides offering the counts of total bacterial cells.

Fluorescence bleaching of BacLight staining

In order to investigate the color fading of stained samples due to the exposure to fluorescence, we compared the pictures of stained bacterial cells taken at the beginning of the exposure and 1 minute later. Figure 2.3 illustrates the results of BacLight-stained *B. subtilis* and *Micrococcus* sp. Both *B. subtilis* and *Micrococcus* sp. samples faded apparently, indicating a significant fluorescence bleaching of stained bacteria due to the 1-minute exposure.

It was found that 62 ± 27% of viable cells and 5 ± 12% of non-viable cells in the

BacLight-stained samples disappeared in 1 minute. The bleaching of BacLight-stained bacteria was very serious in comparison with that of DAPI stained samples in which 7 ± 8% of bacteria faded although the fading could be somewhat delayed by reducing the fluorescence. We found that BacLight-stained bacterial cells had to be photographed with the neutral density filters and within the initial several seconds of fluorescence exposure in order to get pictures suitable for cell counting and statistical analysis. Otherwise, the fading of countable bacterial cells would cause underestimation of bacterial concentration. Notice that the abundance of bacteria in the air is much lower than that in other kinds of media such as lake water or surface soil. A systematic underestimation in counting cells could result in a large uncertainty in the abundance estimate.

Improvement with glutaraldehyde fixation

The stained *B*. *subtilis* and *Micrococcus* sp. of glutaraldehyde fixed samples at the beginning of the microscope fluorescence exposure and after 1 minute exposure were compared (Figure 2.4). The color of *B*. *subtilis* and *Micrococcus* sp. bleached in the 1-minute exposure to fluorescence. However, the bleaching, in particular for green bacterial cells, was apparently slower than that of unfixed samples (Figure 2.3). Statistics of countable bacterial cells in the fixed *B*. *subtilis* samples revealed that about 26 ± 11% of viable cells and -8 ± 21% of non-viable cells in the BacLight-stained samples disappeared in the 1-minute exposure, which were remarkably smaller than the reduction ratios of unfixed samples. Similar improvement by the fixation was not confirmed in the DAPI-stained samples (8 ± 8% of bacterial counts disappeared in the 1-minute exposure), suggesting fixed viable cells of *B*. *subtilis* must be more resistant to fluorescence bleaching than unfixed ones, and the

fixation did delay the bleaching of BacLight-stained bacterial cells.

The enumeration results of viable cells in unfixed and fixed *B*. *subtilis* samples in the three rounds of experiments are summarized in Figure 2.5. On average, the ratios of viable cells to total cells in unfixed samples were 80 ± 6%, 71 ± 12%, and 59 ± 6% at the beginning of fluorescence exposure and were 70 ± 9%, 68 ± 10%, and 4 ± 4% after 1 minute. In contrast, the ratios in fixed samples were 88 ± 6%, 78 ± 7%, and 94 ± 6% at the beginning of exposure and were 82 ± 8%, 73 ± 9%, and 85 ± 7% after 1 minute. Although viable cell counts in fixed samples also decreased due to the fluorescence exposure, the reduction relative to the total cells on average, 7%, was much smaller than that in unfixed samples, 23%. These results indicate that the enumeration accuracy of *B*. *subtilis* treated with the fixation was better than that without the fixation. Fixation with glutaraldehyde can benefit the observation and enumeration of BacLight-stained *B*. *subtilis* cells with epifluorescence microscopy.

The fixation was expected to have protected viable cells from environmental stresses that might injure the cells during the experiments such as osmotic pressure change in filtration and dye washing. Glutaraldehyde is a chemical disinfectant and displays antimicrobial activity. Fixation with this agent can strengthen the membranes of bacterial cells. For gram-positive bacteria such as *B*. *subtilis*, glutaraldehyde can react with the peptidoglycan and produce cross-links in cell walls, resulting in a strengthening and sealing effect on the walls [*Gorman et al.*, 1980]. This mechanism should have worked on the *B*. *subtilis* and strengthened the bacteria against the osmotic change and/or the filtration pressure.

It must be noted that the antimicrobial action of glutaraldehyde differs according to bacteria. Fixation of gram-negative bacteria with glutaraldehyde could cause partial sealing or contraction of the outer layers of the cell envelope and destruct the cells

[*Gorman et al.*, 1980]. It was reported that the counted viable cells of *Escherichia coli* (*E. coli*) were reduced due to fixation with 5% glutaraldehyde in BacLight staining [*Boulos et al.*, 1999]. However, in the same study, *Citrobacter freundii* was not affected by glutaraldehyde, although it also belonged to the group of gram-negative bacteria. In order to test glutaraldehyde-induced effects on viable cells of gram-negative bacteria in BacLight stain, we applied 1% glutaraldehyde fixation to *E. coli* JM109 and *Ralstonia eutropha* JCM 11282T using the same procedures as to *B. subtilis* and *Micrococcus* sp. in the laboratory experiments. Figure 2.6 shows the examples of the stained *E. coli* and *R. eutropha* at the beginning of fluorescence exposure. Fixed bacteria in the microscopic field were obviously clearer in shape than those in unfixed samples. Many bacterial cells in the unfixed samples were ambiguous and uncountable. Moreover, there were approximately 20–30 non-viable cells (red cells) in one field (approximately 3000 μm^2) of unfixed samples, while few were found in fixed samples, in particular in fixed *E. coli* samples. These results indicate that 1% glutaraldehyde fixation is also applicable to some gram-negative bacteria for the improvement of enumeration accuracy in the case of BacLight stain.

Gram-positive bacteria were generally found in the atmosphere by culturing methods, whereas gram-negative bacteria were primarily found by culture-independent methods [*Després et al.*, 2012]. According to the present results, investigation of airborne bacterial abundance with BacLight staining and enumeration is expected to benefit from glutaraldehyde fixation. The fixation can increase the accuracy of cell counts of viable and total bacteria, such as *B. subtilis* to the extent of our experiments. However, we cannot deny the probable presence of cases that there are substantial bacteria in the air whose cell membranes could be severely damaged by the fixation agent within a short time and viable ones would be identified as

non-viable ones. For the sake of detection accuracy of counting viable and non-viable cells in the air, we suggest using the combination of BacLight stain and glutaraldehyde fixation treatment in parallel with experiments without the treatment in field measurements. The confidence of results on bacterial abundance and viability can be adequately ensured with this combination.

2.3.2. Field test

Figure 2.7 shows examples of the pictures of unfixed and fixed open-air samples stained with BacLight stain and fixed samples with DAPI stain. The samples actually consisted of not only bacteria but also other particulate matters such as mineral particles, soot and droplets. Those particles were distinguishable from bacterial cells according to their irregular and aggregate morphology and fluorescent color. For example, mineral particles usually in irregular shape looked yellow by BacLight stain and greenish yellow or white by DAPI stain [*Hara and Zhang*, 2012; *Hara et al.*, 2011]. In contrast, bacteria were usually spherical and had a size close to or smaller than 1 μm in diameter, which was consistent with the reported size of bacteria in surface soil and sea-water [*Roszak and Colwell*, 1987]. Some particles in Figure 2.7 were dust particles and soot particles. In the atmosphere, there are particles in spherical shape with a size close to 1μm besides bacteria. They are mainly droplets of salt solution or soot spheres and do not contain fluorescent components [*Seinfeld and Pandis*, 1998].

The statistics on the bacterial abundance in the air are summarized in Figure 2.8. In comparison with the DAPI staining, BacLight staining with fixed samples detected the total bacterial counts very well and the detection efficiency was 102 ± 11% (Wilcoxon signed rank test with DAPI staining: P = 0.844). The efficiency of the

staining with unfixed samples was 87 ± 6% (Wilcoxon signed rank test with DAPI staining: $P = 0.031$), which was actually not bad. The detection of staining with fixed samples was always a little more efficient than that with unfixed samples (Wilcoxon signed rank one-side test with unfixed samples: $P = 0.016$), indicating the fixation of bacteria in the samples with glutaraldehyde did improve the efficiency and accuracy of counting total bacterial cells. Therefore, BacLight stain coupled with glutaraldehyde fixation could be applied instead of DAPI staining to identify total bacterial cells in the air.

In contrast to the fact that more viable cells were detected in the fixed samples than in the unfixed samples, the counts of non-viable cells in the fixed and unfixed samples were not very different (Wilcoxon signed rank test: $P = 0.438$). The ratio of viable cells in the fixed samples to those in unfixed samples was 127 ± 25% and that of non-viable cells was 105 ± 25%. Similar to the laboratory experiments, fixing bacterial cells in airborne samples with glutaraldehyde improved the detection accuracy of viable cells and, as a consequence, the accuracy of total bacterial cells in the open air. These results indicate that the combination of glutaraldehyde fixation and BacLight staining is suitable for enumerating airborne bacteria in the air. Using this method allows the counts of viable and non-viable bacterial cells as well as the total cell counts.

2.4. Limitation and comparison with conventional methods

When the concentration of bacterial cells in the air is relative low such as less than $4.6 \cdot 10^4$ cells m^{-3}, only one cell in a microscopic filed as we used in this study is expected if the sample collection time is one hour (0.75 m^3 of air). In such cases, it is impossible to correctly estimate the bacterial cell concentration in the air with the

samples. One hour samples are only suitable to cases when the bacterial concentration is more than 10^5 cells m^{-3}. Sample collection time needs to be extended if the anticipated bacterial concentration in the air is lower than 10^5 cells m^{-3} in order to get confidential results. According to published literature in which airborne bacteria were studied with microscopic enumeration and qPCR, the concentration of bacterial cells in surface air is usually about 10^4–10^5 cells m^{-3} or more except for extreme clean areas such as alpine, remote marine and elevated air [*Bauer et al.*, 2002; *Bowers et al.*, 2009; *Bowers et al.*, 2012; *Bowers et al.*, 2011a; *Chi and Li*, 2007; *Cho and Hwang*, 2011; *Hara and Zhang*, 2012; *Harrison et al.*, 2005; *Tong*, 1999; *Tong and Lighthart*, 2000; *Xia et al.*, 2012; *Yamaguchi et al.*, 2012]. In addition, there might be BacLight stain-resistant bacteria in the air. In this regard, the bacteria detected with the BacLight stain should be considered as the low bounds of bacterial concentration in the air.

Table 2.1 shows the comparison of the BacLight stain with conventional DNA-based methods and DAPI stain in the measurements of airborne bioaerosols. DNA-based methods including qPCR can be used in principle to accurately identify and quantify bioaerosols in the air in terms of DNA and its contents. DAPI staining can detect total DNA-containing bioaerosols. BacLight staining can quantify viable and non-viable bacteria in cell number. The methods with fluorescent stain can get the cell concentration in the air but cannot obtain the information on bacterial composition and community.

Recently, instruments and fluorescence sensors, such as waveband integrated bioaerosol sensors (WIBS) and ultraviolet aerodynamic particle sizer (UV-APS), were developed to monitor the real time in situ concentration of bioaerosols in the air. WIBS measures the real time concentration of total bioaerosols [*Kaye et al.*, 2005;

Toprak and Schnaiter, 2013] and UV-APS measures the real time number concentration of live bioaerosols in the air [*Huffman et al.*, 2012; *Huffman et al.*, 2010; *Prenni et al.*, 2009]. Both enable the information on the number size distribution of the bioaerosols. These instruments are much more efficient than methods with fluorescent stain to demonstrate the evolution of bioaerosols in the air. However, the particles measured by the instruments include not only biological ones but also those whose components (e.g. the organic compounds of polycyclic aromatic hydrocarbons) display fluorescence [*Huffman et al.*, 2010]. Moreover, results from these instruments in field studies lack data qualification by comparisons with other approaches, which make the uncertainties in the data unknown. In this regard, the BacLight stain method we propose in this study actually supplies an available way for the inter-comparison of results from these different kinds of approaches, which will largely benefit the studies of bioaerosols in the air.

2.5. Summary

In this study, BacLight stain was tested to enumerate the viable and non-viable cells of airborne bacteria. Two gram-positive bacterial strains, *B*. *subtilis* and *Micrococcus* sp., were used as model airborne bacteria in laboratory experiments. The results were compared with DAPI stain for quality control.

BacLight stain detected 76–112% of total bacterial cells in comparison with DAPI stain. The ratios of viable cells to total cells showed very good consistency in replicates of the same samples, indicating a good accuracy of BacLight stain in counting viable and non-viable cells of the model airborne bacteria. Glutaraldehyde was applied to reduce the effect of fluorescence bleaching due to the exposure to fluorescence at counting or photographing the stained samples. Results showed that

the fixation slowed the color fading of stained cells and improved the detection accuracy of viable and total cells.

Application of the BacLight stain to air samples, in addition to the total bacterial cell counts consistent with DAPI stain, acquired bacterial viability. And the results with glutaraldehyde fixation were more accurate than those without fixation. Since glutaraldehyde may injure the cell membranes of unidentified bacteria when this approach is applied in field studies, we recommend processing experiments of staining and enumerating fixed samples parallel with staining and enumerating of unfixed samples to ensure accuracy.

The method we described here is able to distinguish viable and non-viable cells besides offering the total counts of bacteria in the air in a time resolution of hours, which is the typical time scale of weather changes. The operation procedures are simple, the time resolution is short enough for pursuing bacterial concentration changes under different weather conditions, and no expensive instruments are necessary. Such a method allows the verification of traditional methods and new techniques in measuring live and dead bacteria in the air. The only exception is that the procedures are labor intensive. The advantages enable its wide application in approaching the viability and abundance of airborne bacteria to offer the crucial information for the elucidation of bacterial movement with air and their effects on climate and ecosystems.

Table and figures

Table 2.1. Comparison of the BacLight stain and conventional methods (DNA-based and DAPI staining) in the measurement of bioaerosols

	Bacteria	Other bioaerosols	Remarks
DNA-based method	Bulk DNA concentration	Bulk DNA concentration	DNA of viable ones available with EMA-PCR
DAPI staining	Total cells	Total particles	Stain all DNA-containing particles
BacLight staining	viable and nonviable	no data	Usually effective for gram-positive bacteria

Reprinted from *Murata and Zhang* [2013]

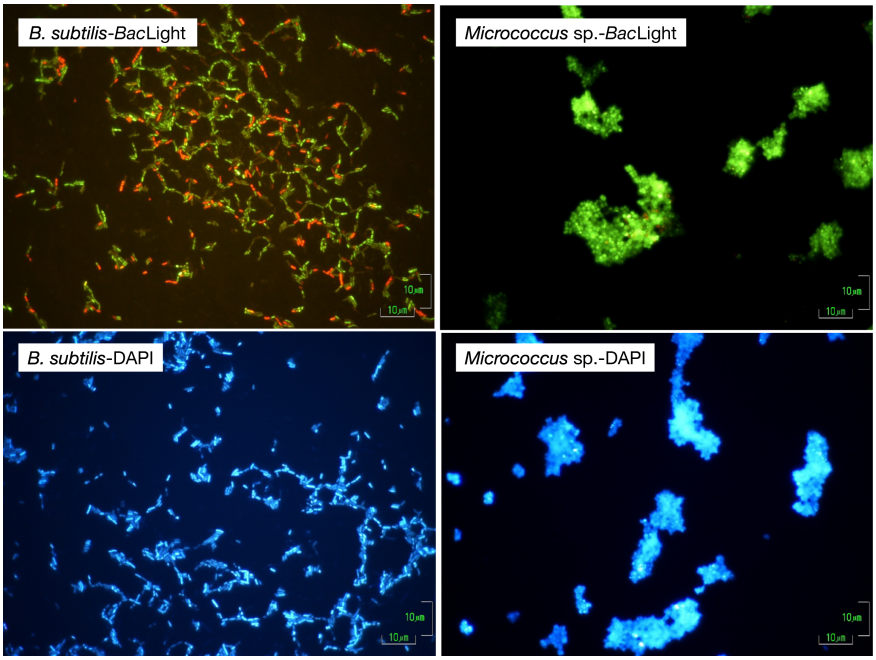

Figure 2.1. *Bacillus subtilis* and *Micrococcus* sp. stained by BacLight stain and DAPI stain. (reprinted from *Murata and Zhang* [2013])

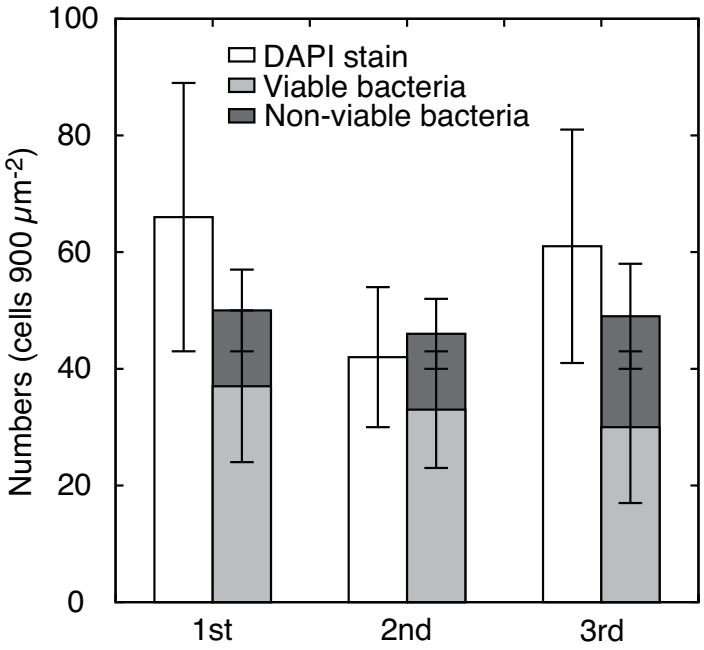

Figure 2.2. Average bacterial numbers in one counted field (900 μm^2) and their standard deviations of BacLight-stained and DAPI-stained *Bacillus subtilis* samples. (reprinted from *Murata and Zhang* [2013])

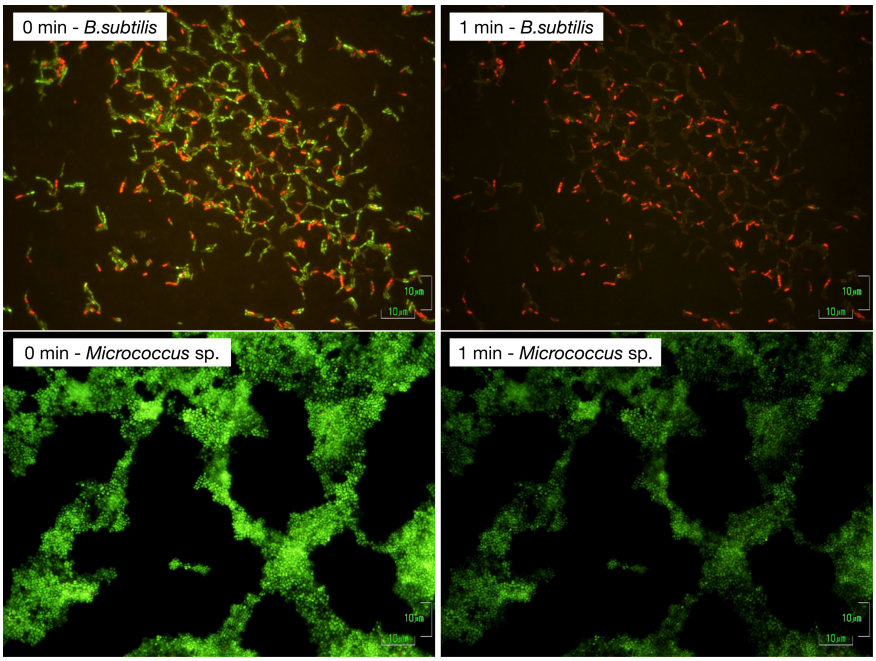

Figure 2.3. *Bacillus subtilis* and *Micrococcus* sp. stained by BacLight stain at the beginning (0 min) and after 1 minute (1 min) of the fluorescence exposure. (reprinted from *Murata and Zhang* [2013])

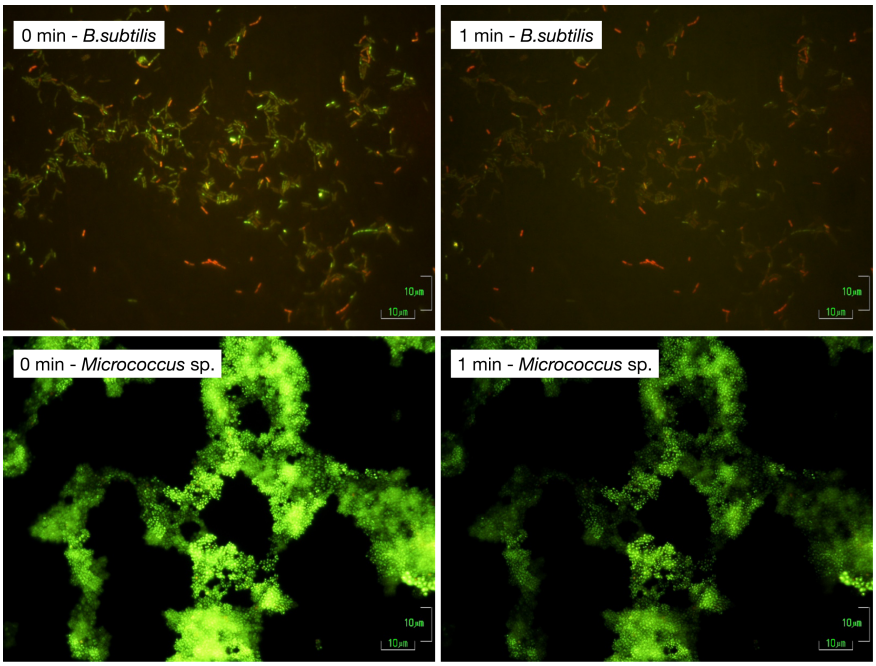

Figure 2.4. Similar to Fig. 3 but for samples with the glutaraldehyde fixation. (reprinted from *Murata and Zhang* [2013])

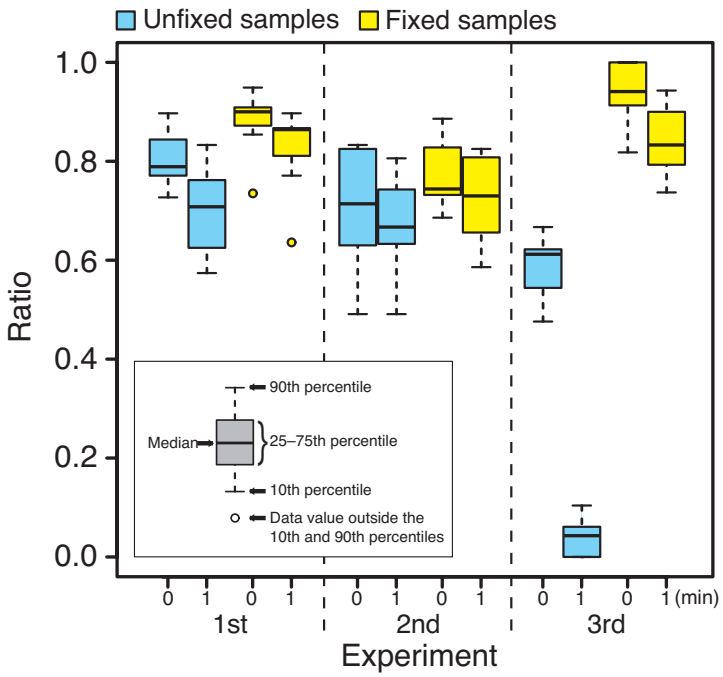

Figure 2.5. Ratios of the viable cells to the total cells in each field (900 μm^2) of *Bacillus subtilis* samples with and without glutaraldehyde fixation at the beginning (0 min) and after 1 minute (1 min) of the fluorescence exposure. (reprinted from *Murata and Zhang* [2013])

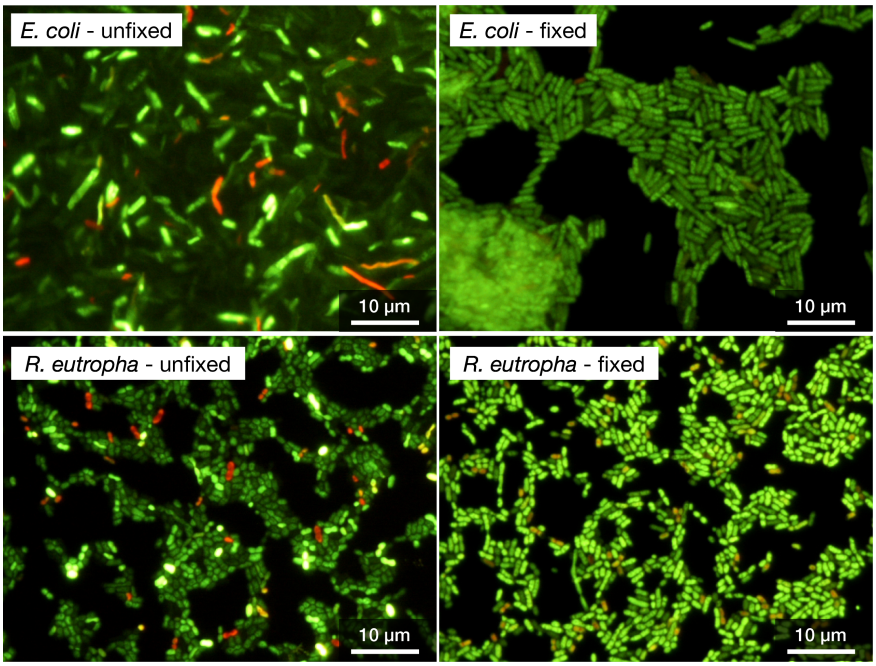

Figure 2.6. Fixed and unfixed *Escherichia coli* and *Ralstonia eutropha* stained by BacLight stain. (reprinted from *Murata and Zhang* [2013])

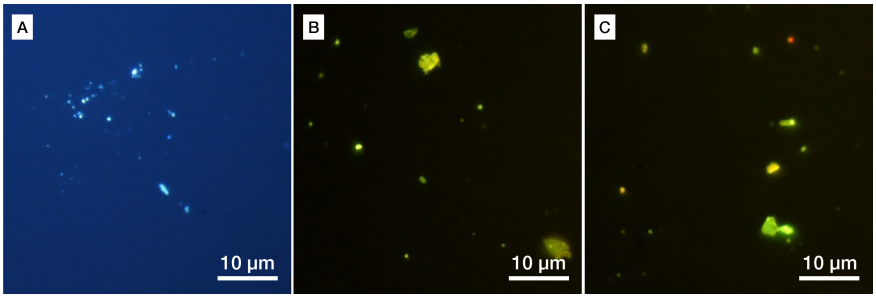

Figure 2.7. Examples of air samples: (A) DAPI stain after the fixation; (B) BacLight stain without fixation; (C) BacLight stain after the fixation. (reprinted from *Murata and Zhang* [2013])

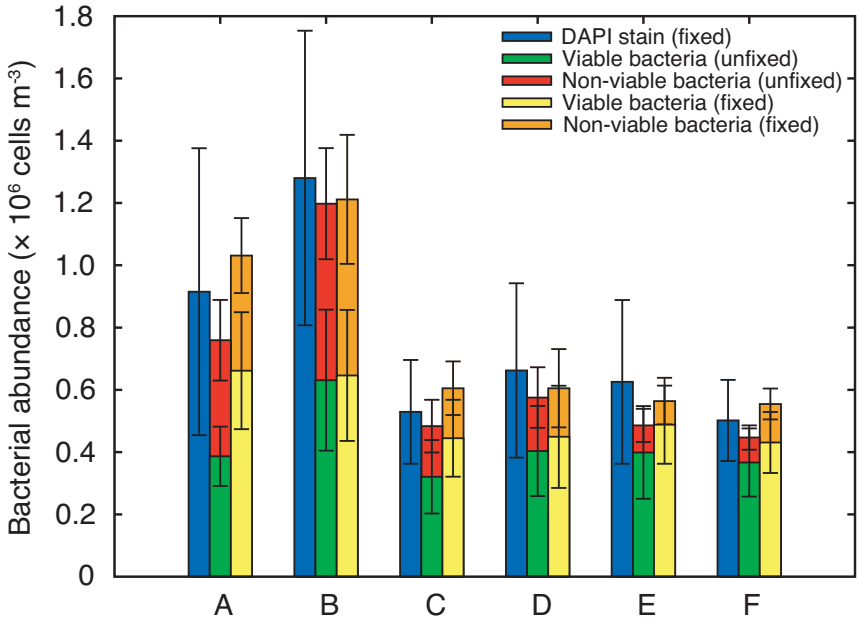

Figure 2.8. Average bacterial abundances in the air and standard deviations identified with the DAPI staining and BacLight staining from the six air samples (marked by A–F). (reprinted from *Murata and Zhang* [2013])

Chapter 3.

Transport of Bacterial Cells toward the Pacific in Northern Hemisphere Westerly Winds: Observations in Kumamoto, Japan

3.1. Introduction

Airborne bacteria constitute a major component of atmospheric biological particles [*Morris et al.*, 2011]. They have a relatively long atmospheric residence time because of their small size, approximately 1 μm or smaller, and can be transported long distances in the atmosphere [*Kellogg and Griffin*, 2006; *Smith et al.*, 2012]. The widespread dispersal of viable bacteria in the air and their settlement to the surface function as links of bacterial communities between geographically isolated regions such as islands or pelagic zones and, consequently, contribute to the development and succession of the microbial communities [*Hervàs et al.*, 2009; *Maki et al.*, 2011; *Womack et al.*, 2010]. Another important fact is that bacteria including viable and non-viable ones and even cell fragments in elevated air can affect cloud development and influence hydrological cycles by enhancing ice nucleation [*Christner et al.*, 2008b; *Möhler et al.*, 2007]. In order to assess these roles and functions, the dispersion of bacteria via wind flow and their biological activity need to be quantitatively evaluated.

In two earlier review papers, *Kellogg and Griffin* [2006] and *Griffin* [2007] demonstrated the global transport of bacteria with atmospheric dust by wind flow. Recent studies showed the remarkable increase of airborne microorganisms with atmospheric dust at the downstream areas of the Asian continent [*Hara and Zhang*, 2012; *Jeon et al.*, 2011; *Wu et al.*, 2004; *Yamaguchi et al.*, 2012; *Yeo and Kim*, 2002]. *Creamean et al.* [2013] found the contribution of bacteria and dust particles after

being transported across the North Pacific to the nucleation of ice clouds over North America. Although it has been gradually recognized that bacteria are widely transported in air parcels such as dust plumes, spatial-temporal variability of bacterial cell concentration and viability in the air under different weather conditions has not been carefully addressed.

Bacteria are constantly released into the air from surfaces such as vegetation, soil and water [*Burrows et al.*, 2009]. The abundance of airborne bacteria is closely dependent on the history of air parcels (e.g. the origin of air parcels and/or where they have passed) and bacterial multiplication in the air [*Fulton*, 1966; *Jones and Harrison*, 2004; *Shaffer and Lighthart*, 1997]. It is usually considered that bacteria in the air hardly multiply because air is a severe environment for bacteria, compared to water and soil except in some specific cases such as cloud droplets or ice crystals [*Sattler et al.*, 2001; *Womack et al.*, 2010]. Without scavenging via precipitation and droplet or ice nucleation, dry deposition will be the only efficient removal process for airborne bacteria. On a certain scale of time or space, the variation or movement of an air parcel can be regarded as an adiabatic process, for which there is rare mass or heat exchange between the parcel and the ambient air except work. The consequences of such atmospheric phenomena are, for example, the frontogenesis and cloud formation in cyclones. Therefore, the abundance and viability of bacteria may be very different in thermodynamically and historically different air parcels.

In the Northern Hemisphere middle latitude regions, the westerly wind flow blows airborne particulate matter from the Asian continent to downstream areas, sometimes to North America and even globally [*Uno et al.*, 2009]. The wind flow advects cyclones and anticyclones eastward, the result of which is the alternative passage of counterclockwise and clockwise vortexes. Such synoptic air movement dominates the

weather in East Asia and governs the export manner of the particulate matter from East Asia for long-range transport [*Jacob et al.*, 2003]. Air within the territory of anticyclone is stagnant, which easily results in the accumulation of air pollutants. In contrast, the passage of cyclones causes rapid alternation of air parcels from warm and humid prefrontal air to cold and dry postfrontal air, accompanied with rapid weather changes. Prefrontal air and postfrontal air within a cyclone arriving at southwestern Japan usually have passed different areas and approach from different directions before arrival. Consequently, airborne particles are expected to be completely different before and after the arrival of a cold front even if they are within the same territory of a cyclone. Airborne particles including bacteria therefore need to be identified separately according to the thermodynamic and historical properties of the air parcels in which they are loaded.

In this study, abundance and viability of airborne bacteria and their evolution associated with cyclones and anticyclones were investigated using a fluorescent staining, LIVE/DEAD BacLight Bacterial Viability Kit, and microscopic enumeration at Kumamoto in southwestern Japan. Kumamoto is located in the coastal area of Kyushu with the East China Sea to its west. Westerly wind flows sweep continentally-originated particulate matter to this area with less influence of local emissions in the Kyushu area except that from ocean areas, which allows the area to be suitable for investigation of particulate matter from the Asian continent. Here we report the bacterial abundance and viability according to synoptic weather conditions and outline how and how many bacteria were transported in the westerly wind flow from the East Asia toward northwestern Pacific.

3.2. Material and methods

3.2.1. Observation site and period

Samples were collected during the passage of five cyclones and nine anticyclones on the balcony (32.806°N, 130.766°E; approximately 20 m above the ground) of a building at Prefectural University of Kumamoto between 12 October 2011 and 7 April 2013 (Figure 3.1). Details of sample collection time and the weather conditions are illustrated in Supplementary Table S1. The surrounding areas of the site are residences. Emissions of local particulate matter that might severely influence the site were not expected.

Samples associated with anticyclones were collected when the weather was governed by anticyclones or was in the transition stage from cyclones to anticyclones (the approaching of anticyclones after the passage of cyclones). We defined these two conditions as (a) anticyclone and (b) approaching anticyclone (surface chart examples in Figure 3.1a and Figure 3.1b). Under such weather conditions, the weather was fine and wind was weak. There was no short-term rapid variation in the air. These samples enabled us to acquire the information on bacterial accumulation in the air. In total, ten samples were obtained, five of which were anticyclone samples and five were approaching anticyclone samples.

Samples during cyclone passage were obtained during five periods: 15–16 October 2011 (front-case 1), 6–7 November 2011 (front-case 2), 22–24 November 2011 (front-case 3), 9–10 March 2013 (front-case 4) and 5–7 March 2013 (front-case 5) when Northern Hemisphere middle latitude cyclones passed the observation site. We defined prefrontal and postfrontal conditions as (c) prefrontal: cyclones were approaching or arrived at the site with their cold fronts to the west of the site, and (d) postfrontal: from the cold front passage to the end of the cyclone coverage (weather

chart examples in Figure 3.1c and Figure 3.1d). In order to get the variation of bacterial cell concentration and viability during the passage of cold fronts, multiple samples were collected with the time interval of three hours or larger. We did not collect samples when it was raining because of the lack of knowledge on wet removal of bacterial cells. For this reason, no samples in the prefrontal air of front-cases 1 and 2 were available. Samples in both prefrontal and postfrontal air were obtained in front-cases 3, 4 and 5. In addition, weak Asian dust occurred in the postfrontal air of front-case 4 according to the weather report of Japan Meteorological Agency.

In addition to the sample collection, size-segregated number concentration of aerosol particles was measured with an optical particle counter (MetOne HHPC-6, Hach Co.). The counter measured the concentration in six size ranges of 0.3–0.5, 0.5–0.7, 0.7–1.0, 1.0–2.0, 2.0–5.0 and >5.0 μm with the time interval of 10 minutes. Weather conditions were monitored with an automatic weather observation system that was set up on the roof of a building next to the observation site. The system recorded temperature, relative humidity, pressure, wind speed, wind direction and global solar radiation every minute, and in this study we used 10-minute averages to document the weather evolution. Due to instrument troubles, data from 10:00 JST (Japan Standard Time) to 19:00 JST on 22 November in front-case 3 and pressure in front-case 4 were not available. Meteorological records by the Kumamoto Meteorological Observatory (approximately 8 km from the observation site) were used to compensate the data loss.

3.2.2. Sample collection and stain

Airborne bacteria were collected with BioSamplers (SKC Inc.). The flow rate was 12.5 L min^{-1} and the collection efficiencies of particles with diameters of 0.3 μm, 0.5

μm, 1.0 μm and 2.0 μm were 79%, 89%, 96% and 100%, respectively [*Willeke et al.*, 1998]. The inside of the samplers was coated with dimethyl polysiloxane (L-25, Fuji systems Corp., Japan) to minimize potential adherence of bacterial cells onto the glass surface. Moreover, the samplers were sterilized at 180°C for 2 hours and washed with particle-free water (pre-filtered through 0.025 μm pore-size filters) before sample collection. The collection liquid media for each sample was 20 mL of phosphate buffer saline (PBS, 0.9%), which had been autoclaved at 121°C for 20 minutes.

The collection time for one sample was 1 hour and bacteria in 0.75 m^3 air was pumped through the sampling PBS. In order to keep the collection efficiency, the PBS was checked and the loss due to evaporation was compensated every 20 minutes. After a sample was collected, the PBS was transferred into a centrifuge tube and adjusted to 25 mL with particle-free water. Then the sample liquid was treated with 1 mL of 25% glutaraldehyde (final concentration: approximately 1%) in a dark place at 4°C for 30 min. Blank control of the sampling procedure was checked by 10 mL of PBS sealed in a centrifuge tube, which had been placed by the BioSampler for every sample, and was treated with the same procedure of the sample.

LIVE/DEAD BacLight Bacterial Viability Kit L13152 (BacLight stain; Life Technologies Inc.) was used to stain the samples. The BacLight stain is composed of two nucleic acid binding fluorescent dyes, SYTO 9 and propidium iodide (PI). SYTO 9 can penetrate all bacterial membranes (intact and injured) and label the cells green. PI can only penetrate injured bacterial membranes and label the cells red with diminishing green stained by SYTO 9. In this study, we considered bacterial cells with injured membranes as non-viable (dead) bacteria and those with uninjured membranes as viable bacteria following previous studies [*Boulos et al.*, 1999]. The staining was conducted with 60 nM of SYTO 9 and 300 nM of PI for 15 min in a cold

dark place. From each stained sample, three sub-samples (each was 8 mL) were prepared on slides with 25 mm-diameter and 0.2 μm-hole black polycarbonate filters for bacterial cell enumeration. In addition, one slide for the blank control sample was prepared in the same way for quality control. The primary application of this method to Asian dust was described by *Hara and Zhang* [2012] and its efficiency with glutaraldehyde fixation in the application to airborne bacteria was confirmed as described in Chapter 2.

3.2.3. Microscopic enumeration of airborne bacteria

Prepared slides of samples and their blank control were observed using an epifluorescence microscope (Eclipse 80i, Nikon corp.). For each sample, the bacterial cell concentration was estimated by counting green (viable) bacterial cells and red (non-viable) bacterial cells in 20 randomly chosen microscope fields of 100 μm × 100 μm area. An example of airborne bacteria labeled with BacLight stain under the microscope is shown in Figure 3.2. The air samples actually contained various particle types besides bacterial cells. Non-bacterial particles were distinguished from bacterial cells according to their irregular shape, aggregate morphology and/or fluorescent color. Yellow or orange ones by the BacLight stain were mineral particles, in contrast to bacteria cells as green or red spherical spots in a size close to or smaller than 1 μm in diameter [*Hara and Zhang*, 2012; *Hara et al.*, 2011; *Roszak and Colwell*, 1987]. The concentration of airborne bacteria in the air was estimated by subtracting blank control counts. It should be noticed that there might be BacLight stain-resistant bacteria in the air and some viable bacteria might lose their viability even in PBS. In this regard, the viability we detected should be considered as the low bounds.

3.3. Results

3.3.1. Bacteria associated with anticyclones

Air parcels in anticyclones moved slowly and approached the site from the northwest, southwest, west or east (Figure 3.1a). Air parcels of approaching anticyclones moved faster and approached the site area from the northwest (Figure 3.1b). The number concentration of viable and non-viable bacterial cells in anticyclone air and approaching anticyclone air was summarized in Figure 3.3. Also shown in the figure are the number concentrations of aerosol particles in the size range of 0.3–1.0 μm (hereafter called fine particles) and >1.0 μm (hereafter called coarse particles).

When anticyclones governed the weather, the total bacterial cell concentration, the sum of viable and non-viable cell concentrations, showed small differences in different anticyclones. The concentration ranged between 2.9×10^5 and 4.1×10^5 cells m^{-3} and the average was 3.7×10^5 cells m^{-3}. The viability, the ratio of viable cells to total cells, was around 80% except 41% on 20 October 2011, and the average was 71%. Fine particle concentration was 3.7×10^7–2.7×10^8 particles m^{-3}, and coarse particle concentration was 6.6×10^5–1.8×10^6 particles m^{-3}. The particle concentration did not show a correlation with the bacterial cell concentration or viability.

In comparison with those in the anticyclone air, bacterial concentration in approaching anticyclone air was a little higher. The concentration ranged between 5.4×10^5 and 9.9×10^5 cells m^{-3} and the average was 7.0×10^5 cells m^{-3}. The viability averaged 73% with the range of 65–90% and was not much different from that of anticyclone.

3.3.2. Evolution of bacteria during cyclone passage

The postfrontal air parcels moved fast and approached from the northwest (Figure 3.1d), except the postfrontal air of front-case 2 which was stagnant from the Korean Peninsula and the East China Sea due to the slow movement of the front. The prefrontal air of front-cases 3 and 4 approached from southwest (Figure 3.1c) and that of front-case 5 approached from southeast, which were typical air movement patterns of prefrontal air in the observation area. The crowded and curved trajectory plots of prefrontal air indicate that the movement was slower and more stagnant than postfrontal air.

Figure 3.4 illustrates an example (front-case 3) of the evolution of bacteria, particles and weather during the passage of cyclones (data of other front-cases in Supplementary Figure S6). As the front was approaching, relative humidity gradually increased and pressure decreased. After the front passage and rain, relative humidity decreased and pressure increased. Temperature had a clear diurnal variation with a decrease at frontal arrival. In both the prefrontal air and the postfrontal air, the total bacterial cell concentration was on the order of 10^5 cells m^{-3} and its variation was small. However, the bacteria were dominated by viable ones in the prefrontal air while by non-viable ones in the postfrontal air. Non-viable bacterial cells remarkably increased with both coarse and fine particles while the concentration of viable bacterial cells did not change considerably in the postfrontal air in comparison with the prefrontal air.

Table 3.1 is the summary of viable and non-viable bacterial concentrations associated with the passage of cyclones. The average concentration of total bacterial cells in the prefrontal air was 1.1×10^6 cells m^{-3} in front-case 3, 5.4×10^5 cells m^{-3} in front-case 4 and 4.6×10^5 cells m^{-3} in front-case 5. After the front passed, the

concentration became 1.2×10^6, 8.7×10^5 and 5.8×10^5 cells m^{-3}, respectively. On average, bacterial concentration in the postfront air was $8.3 \pm 3.2 \times 10^5$ cells m^{-3}. This value was larger than that ($7.2 \pm 3.2 \times 10^5$ cells m^{-3}) in the prefrontal air. The viabilities in the prefrontal air of front-cases 3, 4 and 5 were 75, 72 and 68%, respectively, and were 51, 53 and 81% in postfrontal air. The viabilities in the postfrontal air were smaller than those in the prefrontal air except front-case 5.

The ranges of fine particle concentration in prefrontal air of front-cases 3, 4 and 5 were 6.4×10^7–2.3×10^8, 1.2×10^8–2.2×10^8 and 7.1×10^7–8.7×10^7 particles m^{-3}, respectively. Those in the postfrontal air were 2.8×10^8–3.1×10^8, 2.3×10^8–3.3×10^8 and 5.4×10^7–1.3×10^8 particles m^{-3}, respectively. The passage of the cold front did not lead to a significant change of fine particles in the air. In contrast, the front passage did cause a considerable increase of coarse particles in the air. The ranges of coarse particle concentration in the prefrontal air were 1.2×10^6–2.5×10^6, 3.5×10^6–5.3×10^6 and 1.4×10^6–1.8×10^6 particles m^{-3}, respectively, and those in the postfrontal air were 5.0×10^6–5.1×10^6, 6.9×10^6–1.2×10^7 and 1.2×10^6–4.7×10^6 particles m^{-3}, respectively. Non-viable bacterial cell concentration synchronously varied with fine and coarse particles in the postfrontal air. Consequently, total bacterial cells virtually varied with the particles (e.g. 18:00–22:00 JST on 23 November in Figure 3.4).

The concentration of total bacterial cells in the postfrontal air of front-case 1 was 3.0×10^5–7.3×10^5 cells m^{-3} with the average of 4.9×10^5 cells m^{-3} and that of front-case 2 was 7.6×10^4–2.3×10^5 particles m^{-3} with the average of 1.8×10^5 cells m^{-3}. The bacterial viability was 44–84% with the average of 60% in front-case 1 and 64–100% with the average of 84% in front-case 2. The concentrations of fine and coarse particles in front-case 1 were 1.0×10^8–2.6×10^8 and 2.3×10^6–5.3×10^6

particles m^{-3}. Those in front-case 2 were 6.7×10^7–1.3×10^8 and 7.3×10^4–2.2×10^6 particles m^{-3}. The concentration of total bacterial cells in the postfrontal air of the two front-cases was lower than that in front-cases 3, 4 and 5. The correlation between the bacteria and coarse and fine particle concentrations was also detected.

3.4. Discussion

3.4.1. Dependence of bacterial abundance and viability on air parcels

The common characteristic of anticyclone air parcels was the very slow movement, suggesting the favor of particulate matter accumulation and a probably significant contribution from the areas close to the site. It was noticed that the abundance was similar to or lower than other weather conditions, indicating less contribution of bacteria by long-range transport and lower bacterial abundance in the mixture of local air and marine air. Limited literature reported that bacterial concentrations in coastal and marine air were ranged from 10^4 to 10^5 cells m^{-3} [*Aller et al.*, 2005; *Cho and Hwang*, 2011], which was less than the concentration of this study by half to one order, suggesting bacteria from local and marine areas were impossible to result in large change of the bacterial abundance we observed, in particular, in the postfrontal air.

The postfrontal air moved fast from the Asian continent to the site and particulate matter in the air parcels would not have been influenced significantly by the areas they passed. In some cases, bacteria in the air were dominated by those from the continent [*Maki et al.*, 2013]. The low concentration of bacteria and suspended particles in the postfrontal air of front-case 2 indicates that the stagnant postfrontal air contained fewer bacteria than that in the postfrontal air of fast-moving fronts. In summary, airborne bacteria in the postfrontal air were categorized into two origins:

continental origin (continentally natural and anthropogenic) and mixture of continental and local origins (island natural and marine). The movement of the prefrontal air of front-cases 3 and 4 was slower than the postfrontal air, suggesting the different source areas and histories of the particulate matter in the prefrontal air from those in the postfrontal air. The bacteria in the prefrontal air were more likely a mixture consisting of those from both the marine areas between China and Japan and the populated continental areas of eastern China. The air parcels of approaching anticyclone were the intermediate between postfrontal and anticyclone air. Continentally originated air mass was more predominant in these air parcels, compared with anticyclone air. This is consistent with the fact that the concentration of airborne bacteria was higher than that in anticyclone.

Fast-moving postfrontal air parcels brought more bacteria from the continent, while the viability was lower than those more influenced by local and regional emissions. Similar results were reported by *Hara and Zhang* [2012] in case studies of Asian dust when air parcels loading dust particles arrived at the site of this study in postfrontal air. They argued that the concentration of viable bacteria decreased during inter-continental travel. Higher bacterial viability in anticyclone air parcels indicates more viable bacteria from local sources than from remote sources. Decrease of the viability was especially large when non-viable bacteria increased just after the end of rain (e.g. 18:00–22:00 JST on 23 November 2011 in Figure 3.4). This is naturally acceptable because bacteria from local or regional sources had a short residence time and their exposure to the environment stress was much less than those in the postfrontal air.

3.4.2. Dependence of bacteria on particles

The abundance of viable and non-viable bacteria versus the concentration of fine and coarse particles in different air parcels is shown in Figure 3.5. Bacteria in the postfrontal air had a clear correlation with the particle concentration. Moreover, non-viable bacteria were more sensitive to coarse particles than viable ones. It is noted that the increase of non-viable bacteria was larger than that of viable ones in the postfrontal air (Figure 3.5). Bacterial viability decreased with the increase of particles in the postfrontal air (Figure S7). However, the bacterial abundance and viability in anticyclone and prefrontal air did not have a correlation with particles.

At southwestern Japan, front-associated increase of coarse particles frequently has a time lag from the increase of fine particles, especially when postfrontal air brings Asian dust particles [*Zhang et al.*, 2006]. Maximum concentration of fine particles occurs before the arrival of cold fronts while that of coarse particles after the arrival. This is attributed to the separated status of prefrontal air and postfrontal air. Particulate matter in prefrontal air is substantially influenced by anthropogenic emission in eastern China while those in the postfrontal air frequently contain fewer anthropogenic pollutants but more natural ones such as dust particles. The correlation between bacterial abundance and particulate matter in the postfrontal air was more likely a dependence of bacteria on coarse particles rather than on fine particles although there was a correlation between bacteria and fine particles. There are two reasons for excluding the possibility of a correlation between bacteria and fine particles. One is that a large part of fine particles should have been produced via the homogeneous processes in the atmosphere and the processes had no direct relation with pre-existing particles [*Seinfeld and Pandis*, 1998]. Another is that the correlation in the figure is likely a false phenomenon because coarse particles and fine particles

had a weak correlation. There are usually primary particles among fine particles and the increase of coarse particles is frequently accompanied with the increase of fine particles in East Asia. This hypothesis is supported by the dependence of airborne bacteria on dust particles [e.g., *Maki et al.*, 2013; *Yamaguchi et al.*, 2012].

Bacterial viability in the air was considered to be sensitive to meteorological factors and atmospheric pollution [*Chi and Li*, 2007]. We have only local weather conditions and our results did not show a dependence of bacterial viability on them including temperature, relative humidity and solar radiation in the anticyclone and prefrontal air (Figure S8). It has been found that bacteria frequently adhered to aerosol particles and/or incorporated into water droplets [*Polymenakou*, 2012]. Such a combination could protect the bacteria from ultraviolet radiation, dehydration and desiccation stresses in the atmosphere [*Kellogg and Griffin*, 2006; *Polymenakou*, 2012]. Although current techniques cannot quantitatively identify bacteria adhered to aerosol particles, the lower viability in the postfrontal air and the correlation of bacterial viability with coarse particles suggest that the lower viability was more likely due to the origin of the bacteria rather than a reduction of viability in the air.

3.4.3. Transport manner of bacteria from East Asia toward northwestern Pacific

In the middle latitudes of both hemispheres, air is constantly blown eastward by westerly winds. Any particulate matter which has a long residence time in the air can be transported long distance in the westerly wind flow [*Bates et al.*, 2006]. So far, evidence for the inter-continent transport of microorganisms has been documented mostly in cases when dust particles were detected [*Hua et al.*, 2007; *Jeon et al.*, 2011; *Prospero et al.*, 2005; *Yamaguchi et al.*, 2012]. Dust loading did increase bacteria abundance in the parcels, which made the detection of microorganisms in dusty air

easier than in dust-free air. However, airborne bacteria may also be transported inter-continentally without dust [*Smith et al.*, 2012]. Our results indicate that the action of air parcels transporting bacteria in the westerly wind flow was not dependent on dust loading in the air parcels but on the parcels' thermodynamic properties. Under non-dust conditions, fast-moving postfrontal air parcels retained bacteria and carried them to downstream areas while slowly-moving air parcels such as anticyclones did not move adiabatically to enroll the bacteria within the air parcels without exchanging with ambient environment. Hence, air parcels following fast-moving cold fronts, i.e. postfrontal air parcels, are the most efficient conveyors of bacteria including both viable and non-viable ones from the Asian continent toward the Pacific.

The abundance of bacteria in the postfrontal air parcels we observed was on the order of 10^5 cells m^{-3} or larger. This value is similar to the level of airborne bacteria in dust-free air estimated by previous studies in northwestern China [*Kakikawa et al.*, 2008] and at the same site of this study [*Hara and Zhang*, 2012]. The 10^5 cells m^{-3} range is also in the same order of bacteria in the outdoor urban air in the United States [*Bowers et al.*, 2011b] and even in the same order of that observed in elevated dust-loading air [*Yamaguchi et al.*, 2012]. The similar range of bacterial concentrations in spatially and temporally different air parcels indicates dust-free postfrontal air parcels also bring substantial bacteria to the downstream areas. The transport of bacteria by one dust-free postfrontal air parcel is not likely as efficient as a dust air parcel in the point of view of bacterium loading. However, cyclones occur in the westerly wind flow and pass the Asia continent during all seasons. The postfrontal air therefore can be a constant conveyor of bacteria from the continent to the downstream areas. Such air parcels can frequently move from Asia to the North America quasi-conservatively in spring and autumn, to the polar region in summer,

and to subtropical areas in winter. In this regard, studies on the long-range transport of airborne bacteria should not be limited within the scope of dust-associated air movement. Understanding of the temporal evolution and spatial manner of the global bacterial dispersion in the atmosphere will benefit largely from more broad investigation with careful consideration of weather conditions and air parcel properties.

3.5. Conclusion

In this study, bacterial concentrations and their viabilities were measured under different synoptic weather conditions: anticyclone, approaching anticyclone, prefrontal air and postfrontal air. Results showed that the abundance and viability of bacteria in historically and thermodynamically different air parcels were different, but their cell concentrations were all in the same order of 10^5 cells m^{-3}. The concentration of total bacterial cells in postfrontal air was the highest followed by prefrontal air, approaching anticyclone and anticyclone. Continentally originated and fast-moving air parcels had higher concentration of bacteria than stagnant air parcels. The bacteria in stagnant air were more likely a mixture of long-range transported and regionally or locally originated ones. The viability decreased with the increase of coarse particles in postfrontal air, and the viability in fast-moving postfrontal air was lower than that in stagnant air. No correlation between the viability of airborne bacteria and local meteorological conditions was confirmed. The lower viability of bacteria in the postfrontal air was attributed to the continental origin of the bacteria.

These results indicate constant and widespread transport of bacteria from Asian continent toward northwestern Pacific by postfrontal air and partly by approaching anticyclones air. Even within the same territory of a weather system such as cyclones,

the abundance and viability of bacteria might be very different due to the difference of thermodynamic natures and coarse particle loading.

Table and figures

Table 3.1. Viable/non-viable bacterial concentrations in prefrontal and postfrontal air (reprinted from *Murata and Zhang* [2014])

Cases	Prefront (10^5 cells m^{-3})		Postfront (10^5 cells m^{-3})	
	Viable	Non-viable	Viable	Non-viable
Front-case 1 (15–16 Oct 2011)	n/a*	n/a	3.0 ± 1.2 (20:00 15 Oct–21:00 16 Oct, N = 9)	1.9 ± 0.83
Front-case 2 (6–7 Nov 2011)	n/a	n/a	1.5 ± 0.43 (21:00 6 Nov–22:00 7 Nov, N = 9)	0.30 ± 0.22
Front-case 3 (22–23 Nov 2011)	7.9 ± 1.5 (10:00–16:00 22 Nov, N = 7)**	2.7 ± 0.84	5.9 (18:00–22:00 23 Nov, N = 2)	5.9
Front-case 4 (9–10 Mar 2013)	3.9 ± 0.77 (18:30 9 Mar–13:00 10 Mar, N = 7)	1.5 ± 0.48	4.5 (15:00–19:00 10 Mar, N = 2)	4.2
Front-case 5 (5–7 Apr 2013)	3.0 ± 0.81 (12:00–22:00 5 Apr, N = 4)	1.6 ± 0.92	4.6 ± 0.43 (3:00–16:00 7 Apr 7, N = 3)	1.2 ± 0.98

*n/a: not available **N: number of samples

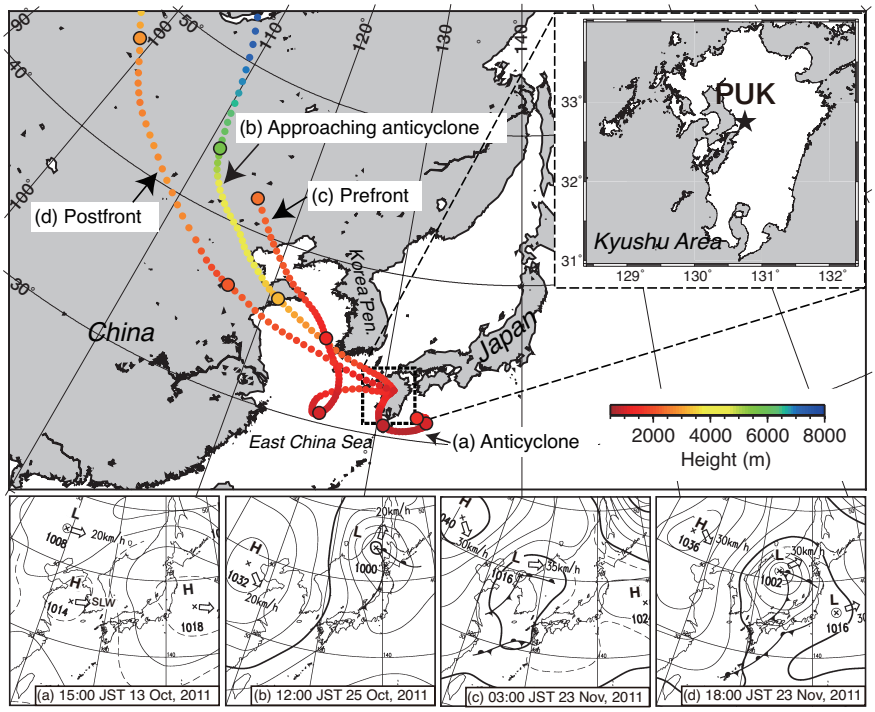

Figure 3.1. Location of the observation site in Kumamoto, Prefectural University of Kumamoto (PUK), and examples of weather charts and backward trajectories: (a) anticyclone, (b) approaching anticyclone (transition region between cyclone and anticyclone), (c) prefront and (d) postfront. The backward trajectories were calculated on-line (NOAA, http://ready.arl.noaa.gov/ HYSPLIT.php). Circles on the trajectories mark 24-h intervals, indicating the typical path, speed and source of the observed air parcels. (reprinted from *Murata and Zhang* [2014])

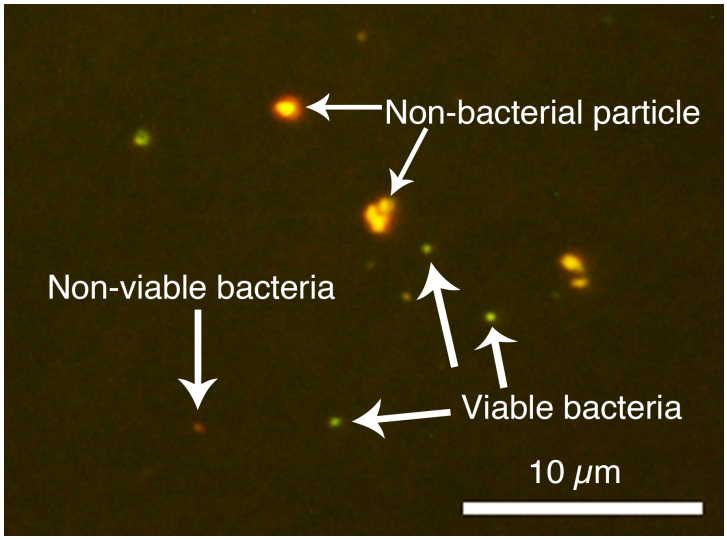

Figure 3.2. Examples of airborne bacteria labeled with BacLight stain. The photograph was taken from the sample of 9:00 JST on 16 October 2011. (reprinted from *Murata and Zhang* [2014])

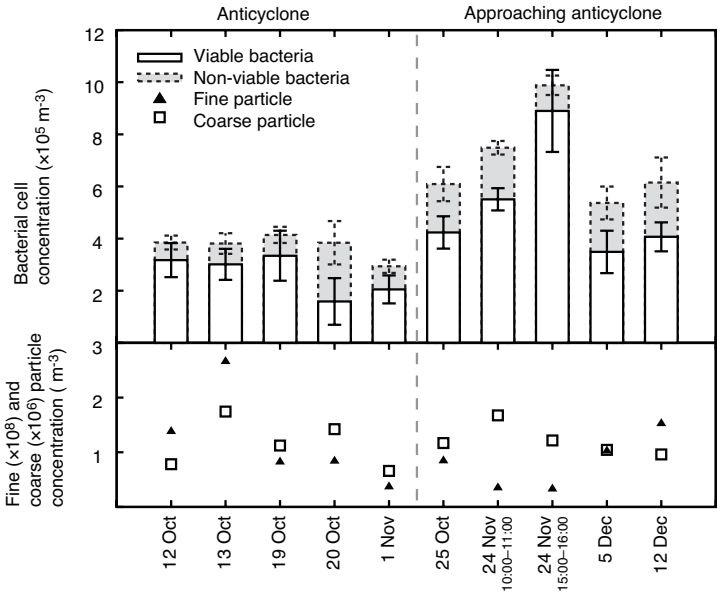

Figure 3.3. Concentration and the standard deviation of viable and non-viable bacterial cells, and concentrations of coarse and fine particles in the cases of anticyclones and approaching anticyclones. (reprinted from *Murata and Zhang* [2014])

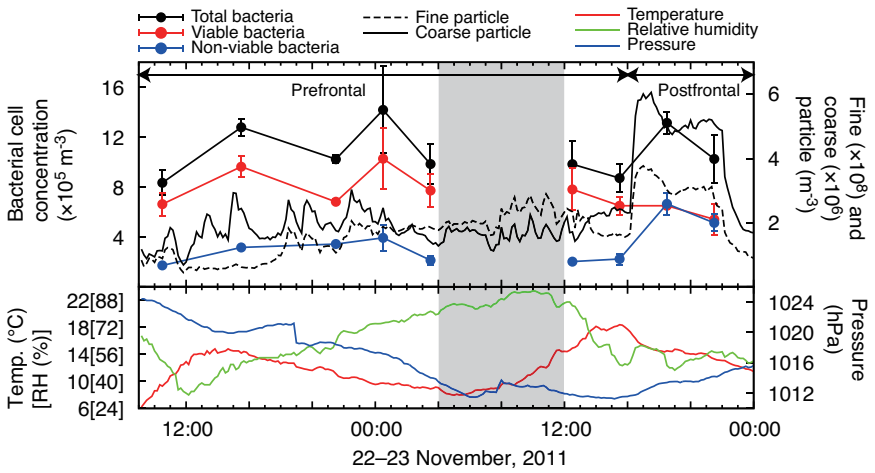

Figure 3.4. Evolution of the concentration of airborne bacteria, and the concentration of fine and coarse particles in front-case 3 from 9:00 on 22 November to 24:00 on 23 November 2011. Temperature, relative humidity and pressure are also shown. The duration of rain is marked by gray shade. (reprinted from *Murata and Zhang* [2014])

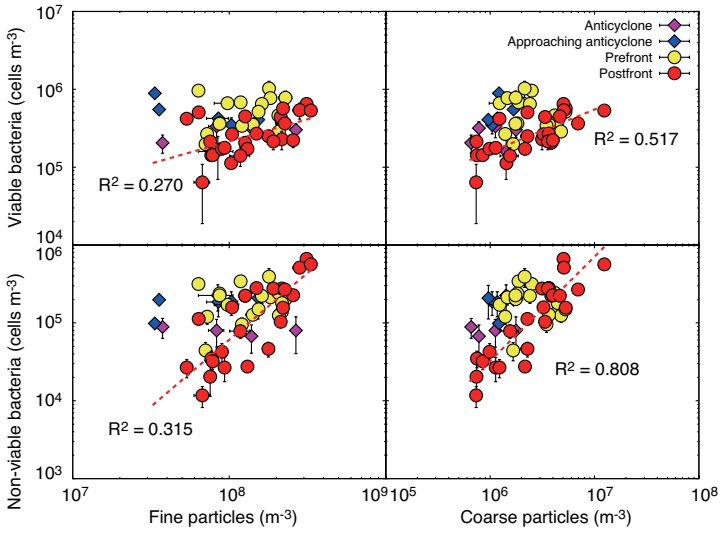

Figure 3.5. Bacteria (viable and non-viable) vs. coarse and fine particles. Error bars for prefrontal and postfrontal concentration mark the standard deviations in three replicated counts of each sample and those of anticyclones and approaching anticyclones were estimated from 20-microscopic fields. The linear regressions for the postfrontal data are marked by red dot lines with correlation coefficients (R^2). (reprinted from *Murata and Zhang* [2014])

Chapter 4.

Concentration of Bacterial Aerosols in Response to Synoptic Weather and Land-sea Breeze at a Seaside Site in Amakusa, Japan

4.1. Introduction

Coastal regions are the boundaries between marine and terrestrial areas and thus have intermediate environmental conditions between the land and the ocean. Seashores can be a substantial source of airborne bacteria due to regular wetting and drying, breaking waves, and wind-generated bubble bursts [*Urbano et al.*, 2011]. The direction of the coastal wind changes daily under stable conditions from onshore during the daytime to offshore during the nighttime [*Stull*, 1988]. This wind exchanges marine and inland aerosol particles, leading to contributions from both marine and terrestrial air to the bacteria in the coastal air. Unfortunately, there are no data available that illustrate the bacterial dynamics in coastal environments although daily wind shift is considered to be a key factor in the exchange of organic and inorganic substances between marine and terrestrial areas [*Dueker et al.*, 2011].

In Chapter 3, the evolution of the concentration and viability of airborne bacterial cells were investigated at a seaside site. We observed the concentration of bacterial cells and their viability when cyclones and anticyclones in the midlatitude westerly wind flows of the Northern Hemisphere alternatively passed the site in the spring. Here, we report on the relevance of synoptic- and local-scale airflows to the concentration and viability of airborne bacteria in coastal zones in the northwestern Pacific.

4.2. Methods

4.2.1. Sample collection

This study was carried out on the platform of a building (32.324°N, 129.993°E, 15 m above the ground) on the seaside of Amakusa Island, Japan, through six sampling campaigns: three associated with the passage of cyclones (22–24 March 2012, 19–21 March 2013, and 15 April 2013) and three with the passage of anticyclones (27–29 April 2013, 10–11 May 2014, and 22–23 May 2014). The site is located on the southwestern coast of Japan, and the north part of the East China Sea is upwind of the midlatitude westerly (Figures 4.1 and S9). The site is frequently affected by air from the Asian continent in addition to local marine and island air. There are few anthropogenic sources of aerosol particles and no nearby industries, except limited agriculture and fishery activities, so this site is suitable for an investigation into the dynamics of airborne bacteria in coastal air under the influence of natural ocean- and island-derived bacteria as well as those that are continental and transported over long distances.

Samples were collected with BioSamplers (SKC Inc.). Before a sample was collected, the sampler was sterilized at 180°C for two hours and then washed with particle-free water (filtered with 0.025-μm pore-size filters and autoclaved). The collection medium for one sample was 20 mL of phosphate buffered saline, which had been pre-filtered with 0.025-μm pore-size filters and autoclaved. The flow rate was 12.5 L minutes^{-1}, and the collection time for one sample was one hour (0.75 m^3 of air). A blank control for each sample was prepared with 20 mL of the medium in a tube, which was placed beside the sampler during sample collection. The frequency of sample collection was about four–eight times per day. We did not collect samples

during rain because any loss due to washout/scavenging could influence the concentrations of bacteria observed in the air.

4.3.2. Fluorescence staining and cell count

After collection, the liquid sample was treated with 1% glutaraldehyde for 30 minutes and stained with LIVE/DEAD BacLight Bacterial Viability Kits (BacLight stain; Life Technologies) for 15 minutes followed by filtration on a 25 mm-diameter and 0.2 μm-hole black polycarbonate filter for bacterial enumeration. Glutaraldehyde was used to minimize cell destruction during the experiment and to prevent photobleaching of bacterial cells under fluorescent field microscopy. The BacLight stain labels bacterial cells with different fluorescent colors according to cell membrane injury; non-viable cells (those with injured membranes) were stained red and viable cells (those without membrane injuries) green. Viability was defined as the ratio of viable bacterial cells (green ones) to total bacterial cells (green and red ones). This is not consistent with standard culture techniques, although culturable bacteria should be uninjured.

A fluorescence microscope (Eclipse 80i, Nikon Corp.) was used to count cells. For each sample and blank, the concentration of bacterial cells was estimated from the number of green (viable) cells and red (non-viable) cells in 20 random 100 μm · 100 μm fields. The cell concentration in the air was calculated from the cell counts following the subtraction of the blank controls. Details about the efficiency of the methodology and observational procedures are described in Chapter 2 and 3.

4.2.3. Particle measurement

During the observation periods, size-segregated concentrations of airborne

particles were measured with an optical particle counter (KC-01D in 2012–2013 and KC-01E in 2014, Rion Co., Ltd); the concentrations of five size ranges were obtained: >0.3, 0.5, 1.0, 2.0, and 5.0 μm in diameter. In this study, fine particles refer to those in the range of 0.3–1.0 μm, and those of >1.0 μm are coarse particles. According to previous study, rapid increases in the concentration of coarse particles on a synoptic scale in the spring are usually caused by the arrival of Asian dust plumes in this area [*Zhang et al.*, 2003].

4.2.4. Meteorological conditions

Meteorological conditions including temperature, pressure, relative humidity, precipitation and wind speed and direction were monitored with a weather transmitter (WXT520, Vaisala). Due to problems with the instruments, data were not available for the periods of 19–21 March 2013 and 10–11 May 2014. Meteorological records from the Ushibuka Meteorological Observatory of the Japan Meteorological Agency (approximately 14.5 km south of the observation site) were used to compensate for the data loss. The weather conditions during the observation periods are summarized in Table S2.

Samples were categorized into four groups based on the synoptic-scale weather and the thermodynamic properties of the air parcels from which the samples were collected: (1) prefront; (2) postfront; (3) approaching anticyclone, which is the transition stage from cyclone to anticyclone; and (4) anticyclone. These categories were determined from surface weather charts and surface barometric evolutions. Details of the categorization were depicted in figure S10. The weather at the site in the spring is usually governed by the Northern Hemisphere midlatitude westerly and characterized by the alternative passage of cyclones and anticyclones. As a cyclone

passed the site, the surface pressure gradually decreased, and the weather became unstable. It frequently rained as the cold front of the cyclone approached the site, and the passage of the cold front was recognized by the surface pressure minimum. After the passage of the cold front, dry and cold air from the Asian continent blew to the site. As anticyclones approached, air descended from the upper layers, and the near-surface wind gradually weakened with the increase in surface pressure. When anticyclones covered the site, the air was stagnant, and the weather was clear. Examples of typical airflows under the different weather conditions are shown in Figure 4.1.

4.3. Results

4.3.1. Passage of cyclones

Figure 4.2 depicts an example of the evolution of the bacterial concentration, the relative concentration of coarse and fine particles, and the weather as a cyclone passed from 19–21 March 2013. As the weather shifted from anticyclone conditions to prefrontal conditions at approximately 00:00 (JST: GMT+09:00) on 20 March, temperature and relative humidity gradually increased while pressure decreased until the arrival of the cold front at approximately 12:00 that day. Bacterial concentration was 7.1–8.9 × 10^5 cells m^{-3}, and the viability was 70–72%. The concentration of coarse particles was approximately 3.7 × 10^6 particles m^{-3}, and the concentration of fine particles varied similarly to that of coarse particles and was approximately 1.7 × 10^8 particles m^{-3}.

It began to rain at approximately 06:00 on 20 March, and the concentration of both fine and coarse particles declined until the rain stopped at approximately 13:00–14:00 the same day. Following the rain, the air became postfrontal, characterized by low

temperature and humidity and a gradual increase in pressure, which was prompted by the arrival of cold and dry air from the Asian continent to the northwest. The concentrations of fine and coarse particles showed peaks of 2.4×10^8 and 8.7×10^6 particles m^{-3}, respectively. The amplitude in the variation of bacterial concentration was small, up to 7.4×10^5 cells m^{-3}, which corresponded to the increase and decrease in coarse and fine particles. In particular, the change in non-viable bacteria correlated more closely with coarse particles than fine particles, and the change resulted in a decrease in viability to 55%.

As an anticyclone approached the site after the passage of a cold front, the weather became clearer with the inverse variations in temperature and relative humidity until the end of the period. The bacterial concentration gradually increased slightly from 4.7×10^5 to 7.1×10^5 cells m^{-3}, and the viability gradually increased from 77% to 87%. In contrast to the increase in bacteria, the amount of fine and coarse particles decreased or did not change considerably, indicating that the bacteria were not correlated with the particles.

During the passage of the cold fronts of the other two cyclones, the bacteria and particles followed similar trends and correlations (Figures S11 and S12). The increase in coarse particle concentration from 22–24 March 2012 was one order of magnitude greater than that during the other cyclone periods, likely indicating a high concentration of Asian dust particles in the postfrontal air. The bacterial concentration, especially non-viable cells, was also higher and reached 10^6 cells m^{-3}, corresponding to the increase in the coarse particles.

4.3.2. Passage of anticyclones

Diurnal variation in local wind was observed when the weather was governed by

anticyclones and was characterized by northerly land breezes during the nighttime and southerly–northwesterly sea breezes during the daytime. Figure 4.3 depicts the evolution of bacterial concentration, particle concentrations, and the corresponding weather conditions during 22–23 May 2014. The vector plots in the figure illustrate wind direction and velocity at 10-minute increments to demonstrate the transition between sea and land breezes. Coarse particle concentrations did not vary greatly and ranged between 1.2×10^6 particles m^{-3} and 4.1×10^6 particles m^{-3}, which were lower than those observed in cyclone periods. Fine particle concentration had a smaller range of $1.1–3.0 \times 10^8$ particles m^{-3} although it was comparable or higher than that with cold front passage, correlating to ambient humidity.

The abundance of bacterial cells was nearly stable at approximately $1.4–3.6 \cdot 10^5$ cells m^{-3} but increased in pulses to $5.7 \cdot 10^5–1.0 \cdot 10^6$ cells m^{-3} at 06:00–07:00, 09:00–10:00, and 21:00–22:00. A bacterial viability value of 73% at the beginning increased to approximately 90% in the pulse period due to the increase in viable cells, and the evolution of the bacterial concentration neither correlated with coarse nor fine particles. A similar trend was observed in the other two anticyclone periods, and the pulse increase in viable bacteria occurred at 06:00–07:00 on 27–29 April 2013 and at 09:00–10:00 and 21:00–22:00 on 10–11 May 2014 (Figures S12 and S14). The amplitude of the pulse increase was approximately two-fold on average.

4.4. Discussion

4.4.1. Long-distance transport of bacteria

On average, the bacterial concentration in the postfrontal air (range: $0.34–1.7 \times 10^6$ cells m^{-3}) was obviously larger than that in the prefrontal air ($3.9–9.0 \times 10^5$ cells m^{-3}). An increase in bacterial concentration was observed after the passage of the cold front

when the amount of coarse and fine particles dramatically increased, and as the bacteria increased, viability decreased due to the presence of comparatively more non-viable and fewer viable bacteria in the postfrontal air. A large increase in non-viable bacteria was confirmed in the dust-load of the postfrontal air, which contained a high concentration of coarse particles, during the cyclone period from 22–24 March 2012. During this time, the total bacterial concentration increased to 10^6 cells m^{-3}, and the viability declined to approximately 23% (Figure S11).

Therefore, the variability in the bacteria in the postfrontal air was dominated by non-viable bacteria and closely related to coarse, and somewhat related to fine, particles (power regression: R^2 = 0.7236 and 0.6397, respectively: Figure S15); no such correlations were found under other weather conditions. This was consistent with previously reported results that fast-moving postfrontal air parcels in Asian continental outflows efficiently conveyed particle-correlated bacterial cells eastward, which is particularly remarkable in substantially dust-loaded air [*Hara and Zhang*, 2012].

It is well known that the postfrontal and prefrontal air in a cyclone are different thermodynamically, and the airborne particles should have different origins and different histories. At a coastal site in China, *Zhang et al.* [2005] demonstrated that the particles in prefrontal air were mainly from anthropogenic emissions and contained more polluted components while the dust particles from desert areas appeared in postfrontal air and were less influenced by anthropogenic pollutants. *Uematsu et al.* [2002] and *Zhang et al.* [2006] also reported on the different natures of the coarse and fine particles associated with prefrontal and postfrontal air along the western coast of Japan during dust and non-dust episodes.

Regarding the correlation between bacteria and particles in postfrontal but not

prefrontal air, bacteria could be expected to be different in the prefrontal and postfrontal air parcels. As a result, airborne bacteria could be regarded as being similar to airborne particles, both biological and non-biological, in cases when they were transported long distances by cyclones. Therefore, the dynamics of airborne bacteria follow the alternation of air parcels associated with the passage of cyclones, which is a major transportation pattern of aerosol particles at sites downwind of the Asian continent [*Liang et al.*, 2004].

4.4.2. Local effects under anticyclones

Pulse increases in bacteria were observed when the wind altered between sea and land breezes. Figure 4.4 illustrates the diurnal evolution of the bacterial concentration in the air and wind speed and direction when the weather was dominated by anticyclones. More than 70% of the bacteria were viable (Table S2), indicating stronger influence of viable bacteria likely from local sources, rather than non-viable ones that had been long-distance transported. Alternating sea and land breezes, which are the general local wind patterns in coastal areas, are prompted by the thermodynamic differences between land and sea [*Miller et al.*, 2003]. They usually occur under anticyclone conditions due to weak synoptic flows, strong solar heating during the daytime, and radiative cooling during the nighttime under cloudless skies [*Flocas et al.*, 2009].

The geographical status and meteorological conditions around seaside sites under anticyclones favor the occurrence of sea and land breezes (the details were described in the supporting information and Figure S16), and it has been found that a sea breeze can regulate air pollution in coastal cities because pollutants are usually restricted to the shallow near-surface layers of air in the breeze, the internal boundary layers

[*Pillai and Moorthy*, 2001]. A study conducted at a coastal site in Greece found an association among ozone, elevated PM_{10} and sea breeze development. In particular, pulsed increases in PM_{10} coincided with sea breeze onset and cessation, and the increase at the onset of a sea breeze was attributed to the weak vertical diffusion of particles through the shallow internal boundary layer that developed with the breeze [*Papanastasiou and Melas*, 2009]. However, the concentrations of coarse and fine particles at the seaside site in this study did not show such evolutions (Figures 4.3, S13, and S14); there was no significant source of airborne particles associated with industries and traffics around the site, although bacteria could be continuously released from the foreshore and the vegetation. Similar to PM_{10} in the Greek research, the accumulation of bacteria emitted from local sources could lead to the pulse increase in bacteria at the onset of a sea breeze.

There was also a pulse increase in bacteria during land breezes from 10–11 May 2014 and 22–23 May 2014. During those times, the wind was very weak, and a large dilution of particles was not expected (Figure 4.4). In addition, it was just after sunset at that time, and a shallow nocturnal layer started to be developed at the site, favoring the accumulation of locally emitted particles in the air. On the other hand, a pulse increase at sunset was observed on 29 April under east-northeast airflows. There were residential sites, agricultural fields, and vegetation areas east and northeast from the site (Figure S9). Relatively high concentration of bacteria might be conveyed by the east-northeast airflows. Therefore, the pulse increase in bacteria at the seaside site was considered to be likely the result of locally emitted bacteria accumulating in stagnant air under either a sea breeze or a land breeze.

4.5. Summary

Bacterial cell concentration and viability were investigated at a seaside site on the coast of southwestern Japan under different weather conditions. Bacterial abundance in prefrontal and postfrontal air was similar, but viability was different. Bacteria with low viability in the postfrontal air were closely linked to aerosol particles, but the stability of anticyclone air favored the accumulation of locally emitted bacterial cells in the coastal air and resulted in pulsed increases in bacteria on the order of 10^6 cells m^{-3} during the alternation of sea and land breezes. These results indicate that the concentration of bioaerosols at the rural coastal site is regulated by the passage of cyclones and anticyclones on a synoptic scale but also by local sea and land breezes under the stable weather conditions of anticyclones.

Figures

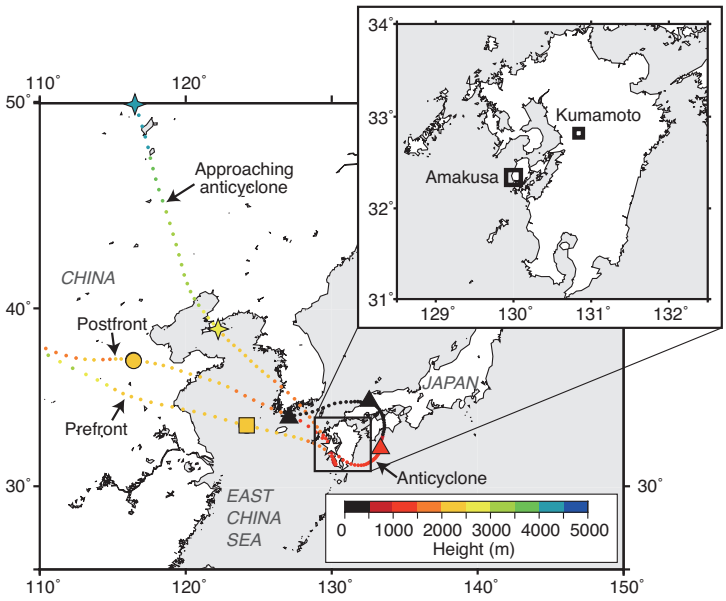

Figure 4.1. Location of the sampling site, Amakusa (marked by a star), and examples of backward trajectories of air parcels for each sample category. The trajectory that began at 0:00 on 20 March 2013 was used for the prefront case; that at 21:00 on 20 March 2013 was used for the postfront case; that at 18:00 on 27 April 2013 was used for the approaching anticyclone case, and that at 9:00 on 11 May 2014 was used for the anticyclone case. Dots in the trajectories indicate 1-hour intervals, and marks (squares, circles, four-pointed stars, and triangles) on the trajectories indicate 24-hour intervals and show the speed of the air parcels. The altitudes of the air parcels along the trajectories are depicted by colors. The HYSPLIT model was used to calculate the trajectories (https://ready.arl.noaa.gov/HYSPLIT_traj.php). The position of Kumamoto is also marked for the following discussion. (reprinted from *Murata and Zhang* [2016, *in submission*])

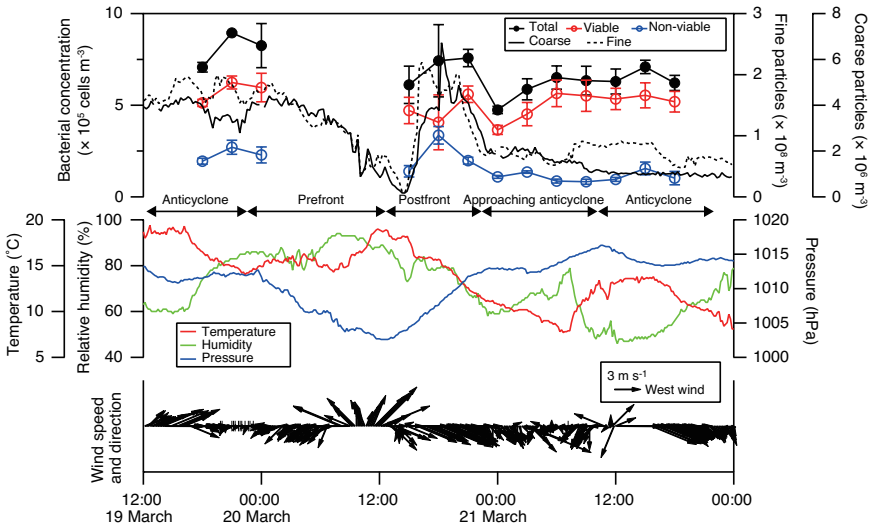

Figure 4.2. Evolution of the concentrations of airborne bacteria and fine and coarse particles from 12:00 on 19 March 2013 to 0:00 on 22 March 2013 (cyclone case). Temperature, relative humidity, pressure, and wind vectors are also shown. (Created based on the data from *Murata and Zhang* [2016, *in submission*])

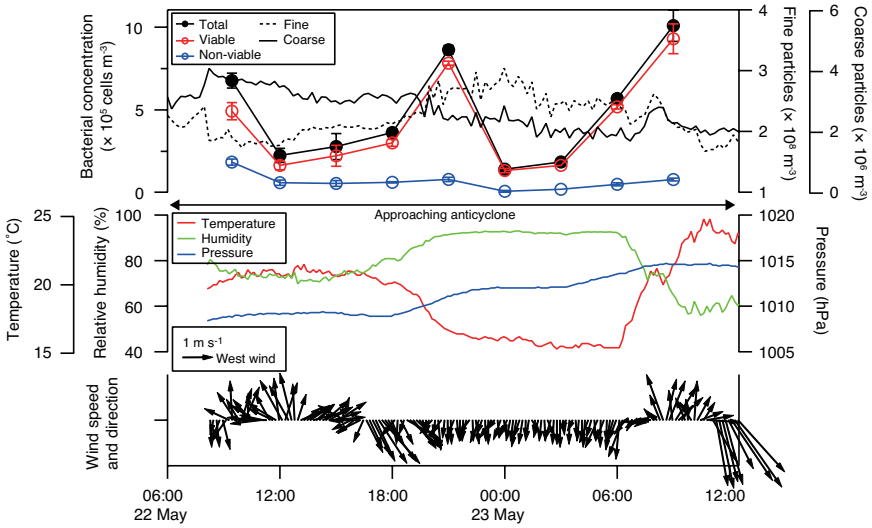

Figure 4.3. Same as in Figure 4.2 but for the case from 6:00 on 22 May 2014 to 12:00 on 23 May 2014 (anticyclone case). (Created based on the data from *Murata and Zhang* [2016, *in submission*])

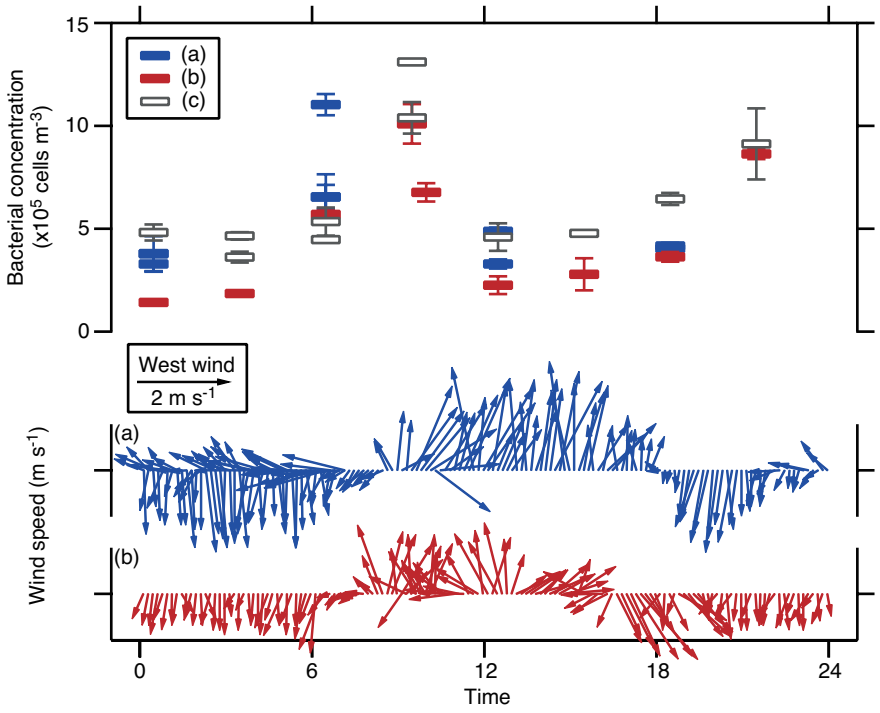

Figure 4.4. Summary of the diurnal evolutions in bacterial concentrations under anticyclonic weather conditions: (a) 27–29 April 2013, (b) 22–23 May 2014, and (c) 10–11 May 2014. Data from different days are merged and plotted by local time. Diurnal evolutions in bacterial concentration are shown in the top panel, and those in wind speed and direction are shown as vectors. Wind data of (c) is not shown because of instrumental errors. (reprinted from *Murata and Zhang* [2016, *in submission*])

Chapter 5.

Remarks and Summary

5.1. Remarks

In this study, we investigated the applicability of the BacLight staining method to the quantification of viable and non-viable bacteria in ambient air. We confirmed that the method could be used for pursuing the evolution of viable and non-viable bacteria in the ambient air with weather changes.

The method can provide broad data for the comparisons with and quality control of online automatic instruments such as WIBS, although the method costs time and intensive labor. Investigations on bioaerosols in the air are being carried out with different approaches, which results in difficulties in comparing and integrating the data. Connecting the monitoring networks on bioaerosols is essential for investigating the role of bioaerosols in the environment. This simple method can gather information on airborne bacterial concentration and viability as standards of quality control for different methods, in addition to the direct application.

Here, to outline the dependence of the abundance and viability of airborne bacteria on synoptic and local weather conditions, we summarize the results at the Amakusa site (seaside) and the Kumamoto site (urban) obtained with the BacLight method. Figure 5.1 shows the average concentrations and viabilities of airborne bacteria at the two sites under different synoptic weather conditions. Despite fluctuations in bacterial concentration along with the changes in airflow at the both sites, the ranges of the concentrations, mostly on the order of 10^5 cells m^{-3}, only differed slightly under different weather conditions. As mentioned previously, the bacterial concentration in the surface terrestrial air has been reported to be on the order of 10^5 cells m^{-3} in

various areas but might increase to 10^6 cells m^{-3} during external transport, such as in the occurrence of Asian dust. The small difference observed in the concentration indicates that 10^5 cells m^{-3} was likely the background airborne bacteria level in the near surface air, even under different weather conditions.

The increase in bacterial concentration when airflow changed (postfrontal air or land/sea breezes under anticyclones) cannot be explained only by local emissions because of the remarkable difference in viability, which was characterized by the correlation between non-viable bacteria and coarse particles in the postfrontal air and by the increase of viable bacteria in the anticyclone air. It has been suggested that bacteria could be injured during transportation through the air because of atmospheric stresses such as UV, desiccation, and air pollutants [*Polymenakou*, 2012]. Low viability in the postfrontal air was possibly the result of the long-distance transport of bacteria, whereas high viability in the anticyclone air was more likely due to local emissions. In addition, the bacterial concentration under anticyclone conditions at coastal areas had pulse increases at the alternation of land/sea breezes. Therefore, the increase in bacterial concentration over the background level of approximately 10^5 cells m^{-3} was promoted by both long-distance transport and local accumulation under land/sea breezes. This means that the bacterial concentration dynamics in the surface air was dominated by airflows on both synoptic and local scales.

The fluctuations in bacterial concentration and viability with synoptic weather were similar at the Amakusa site and the Kumamoto site, but the concentration at the Kumamoto site was higher than at the Amakusa site, except under the anticyclone condition. The average at the Amakusa site was approximately 12–41% less than that at the Kumamoto site under the cyclone condition, whereas it was approximately 39% more under the anticyclone condition. It has been reported that bacterial concentration

is generally higher at urban sites than at coastal sites [*Harrison et al.*, 2005; *Shaffer and Lighthart*, 1997], which was attributed to a greater number of bacterial cells originating from terrestrial sources than from marine sources [*Griffin et al.*, 2006; *Harrison et al.*, 2005; *Prospero et al.*, 2005]. However, the results of this study show that under stable conditions (anticyclones), the accumulation of bacteria at the urban site was weaker than that at the seaside site.

It is not able to discern whether the accumulation of bacteria in urban air is stronger or weaker than in coastal air at this stage. The seaside site in this study is located on a coast with few anthropogenic influences, but there were pulse increases at the onset and cessation of the land/sea breezes. Accumulation in the urban air should be significantly related to anthropogenic activities, which are closely dependent on the population of the city as well as the local industrial and agricultural activities. Bacterial abundance in the air is therefore a consequence of multiple factors and needs to be investigated with careful consideration of weather, surface conditions, and anthropogenic activities.

To demonstrate the similarities and differences among the abundance and distribution of bacteria in various areas and at elevated layers, we further compared the results of this study with data available from the literature (Table 5.1). Airborne bacteria ranged from 10^1 cells m^{-3} to 10^7 cells m^{-3} in different elevated sites. At individual locations, the reported range change has been from one to three orders. Bacteria in terrestrial areas in near-surface air are usually on the order of 10^5–10^6 cells m^{-3}, and the concentration decreases with the distance or altitude from the terrestrial surface, such as in remote areas and the upper troposphere. The bacterial concentration at the onset of land/sea breezes was comparable to the upper boundary of the range, thus indicating a significance of the accumulation and transport

processes associated with the breezes of at least one order. On the other hand, bacterial concentration has been reported to increase by more than one order in dust plumes compared to other weather conditions (Table 5.1). Dust events are intermittent phenomena that occur within one to two weeks of synoptic weather cyclones while land/sea breezes occur daily under proper conditions. Both play major roles in linking geographically different regions in terms of bacterial dissemination. The long-distance transport of dust is a more important pathway for isolated regions while land/sea breezes are more important for coastal regions.

The bacterial concentration in the free troposphere ranges widely, within four orders of magnitude (Table 5.1). These bacteria are considered to be from the surface, and their presence is caused by meteorological and location-specific factors associated with cyclones and typhoons (hurricanes), etc. [*DeLeon-Rodriguez et al.*, 2013; *Kellogg and Griffin*, 2006]. It has been found that sea breezes can lift airborne particles into the free troposphere as well [*Verma et al.*, 2006], and the distribution of bacterial concentrations in the atmosphere is made even by cyclones and anticyclones or, on the other hand, uneven by local emissions, such as those associated with sea breeze. This is an important factor to consider in further studies that assess the effect of airborne bacteria. Compared to dust and soot, bacteria are considered to be less abundant and negligible on the global scale [*Hoose et al.*, 2010b]. However, constant and persistent local emissions of bacterial cells create regional gradients of airborne bacteria and differences in sensitivity on a regional scale. Studies of airborne bacteria dispersal, dissemination, and dynamics on regional and global scales require careful consideration of local and regional emissions and synoptic weather.

5.2. Summary

We confirmed the applicability of the BacLight staining method to the measurement of viable and non-viable bacteria in the ambient air by testing consistencies of the result with traditional DAPI staining in the laboratory and the field. Followed by the methodological tests, changes in bacterial concentration and viability were investigated with the BacLight stain under different synoptic weather conditions in Kumamoto and Amakusa in southwestern Japan. Bacterial concentration did not statistically differ with weather conditions, while viability was significantly different. Non-viable bacteria correlated with particle concentration in the postfrontal air, and lowest viability was observed. In contrast, high viability was observed in the stagnant air associated with anticyclones although the bacteria did not correlatewith particle concentration. These results are consistent with the perspective that bacteria may be injured when they move in the air, and low (high) viability is likely a result of long-distance transported bacteria (locally accumulated bacteria).

The comparison between the results at the Amakusa site and the Kumamoto site showed that bacterial concentration was affected by locations as much as about 40%. The viability did not differ with locations. The comparison with available data from various geographical areas revealed that global range of bacterial concentration in the near-surface air was on the order of 10^4–10^5 cells m^{-3} or 10^5–10^6 cells m^{-3} of magnitude worldwide except for agricultural areas. The range of bacteria in this study was also on the same order of magnitude. Considering that the high concentrations in this study are usually prompted by synoptic and local scale airflows, the dynamics of airborne bacterial abundance and viability governed by synoptic and local weather is crucial to the exploration of the roles that bioaerosols play in the environment via the atmosphere.

Table and figure

Table 5.1. Comparison of airborne bacteria at different geographical sites

Location	Concentration range ($\times 10^5$ cells m^{-3})	Remarks
Amakusa[a]	1.4–13	Passage of cyclones and anticyclones
Kumamoto[b]	2.4–14	Passage of cyclones and anticyclones
	10–160	Asian dust
Urban[c]	3.4–23	No information
Suburban[d]	16	No information
Rural, agricultural[d,e]	0.018–15	Sunny, except grass harvest day
Seaside[f]	0.029–0.14[m]	No information, seasonal mean values
Forest[d]	12	No information
Above sea[g]	0.18–1.2	Unlikely long-range transport
	1.6	African dust
Mountain[h]	0.094–6.1	Clear, cloudy or heavy fog
Free troposphere[i]	0.00043–0.019; 2.1	Sweden; the U.S.
	2.1–20	Japan, Asian dust
Upper troposphere[j]	0.36	Few cloud condition
	0.76–3.0	Hurricanes
Boundary layer[k]	184	Japan, Asian dust
Cloud[l]	0.033–0.18	At a mountain in clouds
	1.23	Over the Gulf of Mexico

[a]The present study, [b]*Hara and Zhang* [2012]; *Murata and Zhang* [2014], [c]*Bowers et al.* [2011b]; *Chi and Li* [2007], [d]*Bowers et al.* [2011a], [e]*Tong* [1999], [f]*Harrison et al.* [2005], [g]*Aller et al.* [2005]; *Cho and Hwang* [2011]; *Griffin et al.* [2001], [h]*Bowers et al.* [2012]; *Xia et al.* [2012], [i]*DeLeon-Rodriguez et al.* [2013]; *Maki et al.* [2013]; *Zweifel et al.* [2012], [j]*DeLeon-Rodriguez et al.* [2013], [k]*Maki et al.* [2013], [l]*Bauer et al.* [2002]; *DeLeon-Rodriguez et al.* [2013]
[m]The concentration enumerated by a Fluorescent In Situ Hybridization (FISH) method but not nucleic acid staining methods such as DAPI.
This table was reprinted from *Murata and Zhang* [2016, *in submission*]

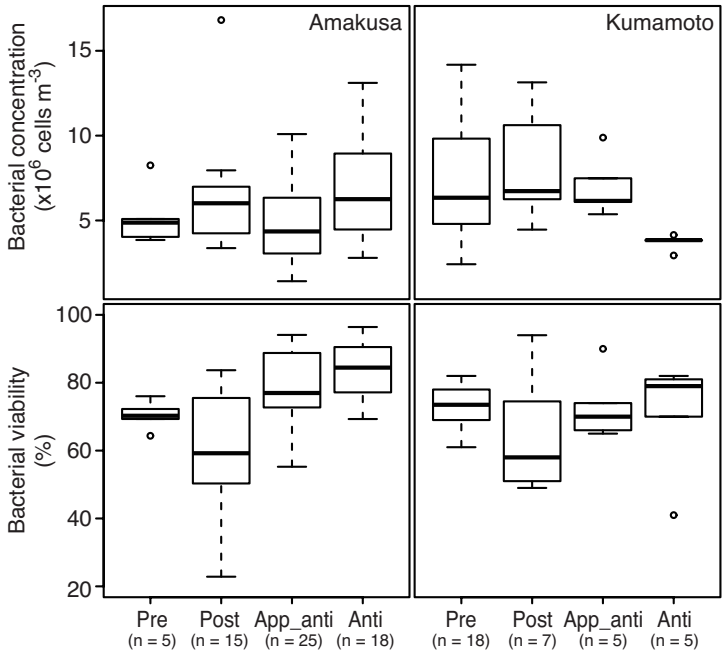

Figure 5.1. Bacterial cell concentration and viability under different weather conditions (prefront, postfront, approaching anticyclone, and anticyclone) at the Amakusa site and the Kumamoto site. Data from different periods are statistically summarized by weather conditions. (reprinted by *Murata and Zhang* [2016, *in submission*])

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Supplementary

Table S1. List of sampling time, weather conditions, and cell concentration detected during the observations at the Kumamoto site [*Murata and Zhang*, 2013]

Sampling time (JST)		Weather	Pressure (hPa)	Temp (°C)	RH (%)	Wind		Bacterial Concentration ($\cdot 10^5$ cells m^{-3})		Remark
Date	Period					Direction	Velocity (m/s)	viable	non-viable	
12 Oct 2011	12:50-13:50	clear	1009.0	27.5	37	N	1.98	3.2	0.67	anticyclone
13 Oct 2011	14:30-15:30	fine	1007.5	27.5	50	W	1.92	3.0	0.80	anticyclone
19 Oct 2011	13:15-14:15	clear	1013.5	25.5	31	WNW	1.32	3.3	0.80	anticyclone
20 Oct 2011	13:15-14:15	clear	1012.1	27.6	44	NNW	2.68	1.6	2.3	anticyclone
1 Nov 2011	13:00-14:00	fine	1016.8	25.7	39	ESE	2.46	2.1	0.89	anticyclone
25 Oct 2011	12:30-13:30	cloud	1010.4	19.2	49	NE	3.97	4.2	1.9	approaching anticyclone
24 Nov 2011	10:00-11:00	fine	1019.8	11.0	42	SW	3.54	5.5	2.0	approaching anticyclone (series 3)
24 Nov 2011	15:00-16:00	fine	1017.6	12.2	35	SW	3.10	8.9	0.98	approaching anticyclone (series 3)
5 Dec 2011	13:00-14:00	clear	1017.8	15.9	41	W	1.49	3.5	1.9	approaching anticyclone
12 Dec 2011	13:45-14:45	fine	1017.0	12.4	45	WSW	1.67	4.1	2.1	approaching anticyclone
22 Nov 2011	10:05-11:05	fine	1024.1	8.6	58	NE	1.1	6.6	1.7	prefront (series 3)
22 Nov 2011	15:00-16:00	fine	1019.9	14.5	50	SW	1.4	9.6	3.2	prefront (series 3)
22 Nov 2011	21:03-22:03	cloudy	1014.2	9.0	80	SE	1.27	6.8	3.4	prefront (series 3)
23 Nov 2011	0:02-1:02	cloudy	1011.6	8.6	84	NE	0.53	10	3.9	prefront (series 3)
23 Nov 2011	3:00-4:00	cloudy	1012.9	10.4	93	WNW	0.55	7.7	2.1	prefront (series 3)
23 Nov 2011	12:00-13:00	cloudy	1011.8	14.8	85	WNW	1.43	7.8	2.0	prefront (series 3)
23 Nov 2011	15:01-16:01	cloudy	1011.4	18.2	52	WSW	2.18	6.5	2.2	prefront (series 3)
9 Mar 2013	18:30-19:30	fine	1009.5	19.6	66	SSW	2.61	4.6	2.4	prefront (series 4)
9 Mar 2013	21:00-22:00	fine	1009.3	18.7	76	S	1.63	3.8	1.4	prefront (series 4)
10 Mar 2013	0:00-1:00	cloudy	1009.5	18.2	78	SSE	1.60	4.0	1.8	prefront (series 4)
10 Mar 2013	3:00-4:00	cloudy	1007.6	18.5	85	SSW	2.28	2.9	1.2	prefront (series 4)
10 Mar 2013	6:00-7:00	cloudy	1008.5	18.4	77	SSW	2.38	3.5	1.3	prefront (series 4)
10 Mar 2013	9:00-10:00	cloudy	1008.6	19.0	74	S	1.98	3.4	0.95	prefront (series 4)
10 Mar 2013	12:00-13:00	cloudy	1007.5	18.5	77	WSW	3.67	5.1	1.5	prefront (series 4)
5 Apr 2013	12:06-13:06	slightly cloudy	1006.8	23.0	29	WSW	2.64	3.7	2.4	prefront (series 5)
5 Apr 2013	15:03-16:03	slightly cloudy	1005.3	23.2	30	SSE	1.05	3.6	2.3	prefront (series 5)
5 Apr 2013	18:02-19:02	cloudy	1004.9	20.8	43	SE	1.43	2.7	1.2	prefront (series 5)
5 Apr 2013	21:00-22:00	cloudy	1004.1	19.2	52	E	1.60	2.0	0.44	prefront (series 5)
15 Oct 2011	20:00-21:00	fine	1005.1	19.7	92	WSW	1.10	2.5	0.46	postfront (series 1)
16 Oct 2011	0:00-1:00	fine	1006.7	18.0	88	S	0.50	4.4	1.0	postfront (series 1)

16 Oct 2011	3:00-4:00	fine	1005.9	17.3	86	W	1.00	2.3	2.8	postfront (series 1)
16 Oct 2011	6:00-7:00	fine	1006.8	16.9	79	ENE	0.54	5.7	1.6	postfront (series 1)
16 Oct 2011	9:00-10:00	clear	1009.1	19.6	51	SE	1.03	2.7	1.6	postfront (series 1)
16 Oct 2011	12:00-13:00	fine	1007.3	22.2	43	WSW	2.20	2.1	2.2	postfront (series 1)
16 Oct 2011	15:00-16:00	fine	1006.8	22.8	42	W	3.07	2.7	2.8	postfront (series 1)
16 Oct 2011	18:00-19:00	fine	1007.8	20.1	54	WNW	1.62	2.1	2.8	postfront (series 1)
16 Oct 2011	21:00-22:00	fine	1008.5	17.4	73	SE	0.55	2.2	2.3	postfront (series 1)
6 Nov 2011	21:07-22:07	fine	1009.5	19.8	87	NW	1.21	0.64	0.12	postfront (series 2)
7 Nov 2011	0:01-1:01	fine	1010.2	18.2	89	NE	0.97	1.4	0.35	postfront (series 2)
7 Nov 2011	3:00-4:00	fine	1010.6	17.3	90	E	0.66	1.1	Not detected	postfront (series 2)
7 Nov 2011	6:01-7:01	fine	1010.8	16.9	87	NE	1.02	1.7	0.28	postfront (series 2)
7 Nov 2011	9:01-10:01	fine	1011.6	21.3	60	ENE	1.94	1.4	0.78	postfront (series 2)
7 Nov 2011	12:00-13:00	fine	1009.8	23.9	46	SSW	3.43	1.4	0.32	postfront (series 2)
7 Nov 2011	15:04-16:04	fine	1009.2	23.8	44	ESE	3.26	2.1	0.20	postfront (series 2)
7 Nov 2011	18:03-19:03	clear	1009.8	20.4	57	ENE	2.22	1.7	0.43	postfront (series 2)
7 Nov 2011	21:05-22:05	clear	1011.0	17.6	68	ESE	1.23	1.8	0.27	postfront (series 2)
23 Nov 2011	18:00-19:00	cloudy	1013.4	14.5	53	W	0.88	6.5	6.7	postfront (series 3)
23 Nov 2011	21:00-22:00	fine	1014.1	13.5	57	NNW	1.95	5.4	5.1	postfront (series 3)
10 Mar 2013	15:00-16:00	cloudy, dust	1007.2	15.5	73	WSW	3.22	5.3	5.6	postfront (series 4)
10 Mar 2013	18:00-19:00	fine, dust	1010.0	13.2	66	WNW	2.41	3.6	2.7	postfront (series 4)
7 Apr 2013	2:50-3:50	cloudy	992.3	8.4	59	W	4.47	4.5	2.2	postfront (series 5)
7 Apr 2013	9:00-10:00	fine	996.8	8.9	52	WNW	5.25	5.0	1.1	postfront (series 5)
7 Apr 2013	15:00-16:00	cloudy	1000.4	10.5	52	WNW	3.12	4.2	0.27	postfront (series 5)

Table S2. List of sampling time, weather conditions, and cell concentration detected during the observations at the Amakusa site [*Murata and Zhang*, 2016, in *submission*]

Sampling time (JST)		Weather	Pressure (hPa)	Temp. (°C)	RH (%)	Wind		Bacterial Concentration ($\times 10^5$ cells m^{-3})		Remark
Date	Period					Direction	Velocity (m/s)	viable	non-viable	
22 Mar 2012	17:19-18:19	Cloudy	1012.9	16.5	59	ENE	1.23	3.3	1.8	Prefront
22 Mar 2012	22:11-23:11	Cloudy	1010.2	17.8	66	ESE	3.27	2.7	1.2	Prefront
24 Mar 2012	3:00-4:01	Cloudy	1006.5	11.1	59	NNW	3.90	3.8	13	Postfront
24 Mar 2012	6:03-7:03	Cloudy	1007.4	10.8	55	NNW	3.73	2.5	3.5	Postfront
24 Mar 2012	7:17-8:17	Fair	1008.4	10.6	52	NNW	4.60	4.7	3.2	Postfront
24 Mar 2012	8:31-9:31	Cloudy	1008.0	11.0	57	NNW	4.23	3.3	2.7	Postfront
24 Mar 2012	9:45-10:45	Cloudy	1008.1	11.8	49	NNW	3.45	2.8	2.7	Postfront
24 Mar 2012	11:00-12:00	Cloudy	1008.5	11.4	53	NNW	3.58	3.9	1.5	Postfront
24 Mar 2012	12:15-13:15	Cloudy	1009.2	10.9	48	NNW	3.13	2.8	0.78	Postfront
24 Mar 2012	15:02-16:02	Cloudy	1009.7	10.0	49	NW	3.78	2.7	0.78	Postfront
24 Mar 2012	18:01-19:01	Fair	1010.8	9.6	43	NNW	2.88	2.8	0.55	Postfront
19 Mar 2013	18:00-19:00	Cloudy	1011.7	16.1	76	W	1.37	5.1	1.9	Anticyclone
19 Mar 2013	21:00-22:00	Cloudy	1012.2	14.8	84	NNE	0.22	6.2	2.7	Prefront
20 Mar 2013	0:02-1:02	Cloudy	1011.1	15.3	85	NE	1.42	6.0	2.3	Prefront
20 Mar 2013	15:00-16:00	Cloudy	1004.8	16.6	79	W	4.92	4.7	1.4	Postfront
20 Mar 2013	18:00-19:00	Cloudy	1008.3	15.2	79	W	5.28	4.1	3.4	Postfront
20 Mar 2013	21:00-22:00	Cloudy	1012.0	12.2	67	WNW	1.58	5.6	2.0	Postfront
21 Mar 2013	0:00-1:00	Fair	1012.9	10.7	60	NW	2.47	3.7	1.1	Approaching anticyclone
21 Mar 2013	3:00-4:00	Fair	1012.4	9.9	67	NW	4.02	4.5	1.4	Approaching anticyclone
21 Mar 2013	6:00-7:00	Fair	1013.8	8.0	72	N	0.77	5.6	0.86	Approaching anticyclone
21 Mar 2013	9:00-10:00	Fair	1015.6	11.8	52	NNE	2.58	5.5	0.83	Approaching anticyclone
21 Mar 2013	12:00-13:00	Fair	1015.2	13.3	48	WSW	4.88	5.3	0.95	Approaching anticyclone
21 Mar 2013	15:00-16:00	Fair	1013.7	13.7	49	W	5.02	5.5	1.6	Approaching anticyclone
21 Mar 2013	18:00-19:00	Fair	1013.5	11.4	59	WNW	3.75	5.2	1.0	Approaching anticyclone
15 Apr 2013	0:00-1:00	Fair	1008.1	16.4	81	ESE	1.45	3.2	1.4	Prefront
15 Apr 2013	3:00-4:00	Cloudy	1007.8	15.2	81	NNW	1.50	3.3	3.3	Postfront
15 Apr 2013	6:00-7:00	Fair	1008.3	14.0	77	NNW	1.03	1.8	2.1	Postfront
15 Apr 2013	9:00-10:00	Fair	1009.7	16.0	55	WNW	1.68	2.0	1.0	Approaching anticyclone
15 Apr 2013	12:00-13:00	Fair	1010.2	16.8	52	E	1.15	2.3	1.6	Approaching anticyclone
15 Apr 2013	15:00-16:00	Fair	1008.8	16.8	49	SSE	2.03	1.0	0.85	Approaching anticyclone
15 Apr 2013	18:00-19:00	Fair	1008.1	16.3	62	SE	1.28	1.5	0.81	Approaching anticyclone
27 Apr 2013	0:05-1:05	Fair	1010.7	15.7	45	NNW	2.35	2.4	1.4	Approaching anticyclone

27 Apr 2013	6:00-7:00	Fair	1012.3	15.4	42	N	2.28	4.8	1.8	Approaching anticyclone
27 Apr 2013	12:00-13:00	Fair	1015.0	17.9	40	E	1.58	2.2	1.1	Approaching anticyclone
27 Apr 2013	18:00-19:00	Fair	1014.7	16.4	45	NNW	1.38	3.2	0.96	Approaching anticyclone
28 Apr 2013	0:01-1:01	Fair	1016.2	10.8	52	N	1.05	3.3	1.1	Anticyclone
28 Apr 2013	6:00-7:00	Fair	1016.7	11.2	48	N	0.77	5.7	1.6	Anticyclone
28 Apr 2013	12:00-13:00	Fair	1017.7	19.7	43	SSW	1.30	3.3	0.97	Anticyclone
28 Apr 2013	18:00-19:00	Fair	1015.6	18.1	62	N	0.47	2.1	0.66	Anticyclone
29 Apr 2013	0:01-1:01	Cloudy	1015.7	16.3	78	ESE	0.70	2.3	1.0	Anticyclone
29 Apr 2013	6:00-7:00	Fair	1014.9	17.9	64	ENE	1.32	9.3	1.8	Anticyclone
29 Apr 2013	12:00-13:00	Fair	1014.6	21.8	61	SSE	4.63	3.7	1.2	Prefront
29 Apr 2013	18:00-19:00	Fair	1011.6	20.8	70	SE	3.62	2.8	1.2	Prefront
10 May 2014	3:10-4:10	Cloudy	1015.9	17.0	53	NE	3.88	4.1	0.50	Anticyclone
10 May 2014	6:00-7:00	Fair	1016.8	17.5	42	ENE	3.98	4.9	0.48	Anticyclone
10 May 2014	9:00-10:00	Fair	1017.7	21.3	40	ENE	3.90	9.8	0.57	Anticyclone
10 May 2014	12:03-13:03	Fair	1016.8	24.6	46	SW	2.62	3.9	0.65	Anticyclone
10 May 2014	15:03-16:03	Fair	1015.8	24.1	49	W	4.25	4.0	0.82	Anticyclone
10 May 2014	18:01-18:07	Fair	1016.2	21.3	57	W	3.07	6.0	0.43	Anticyclone
10 May 2014	21:00-22:00	Fair	1017.7	18.9	70	NNE	0.67	8.5	0.61	Anticyclone
11 May 2014	0:00-1:00	Fair	1017.1	18.5	68	NNE	1.42	4.2	0.60	Anticyclone
11 May 2014	3:00-4:15	Fair	1015.9	18.2	68	NE	3.93	3.1	0.53	Anticyclone
11 May 2014	6:00-7:00	Fair	1016.0	18.0	73	ENE	3.23	4.0	0.42	Anticyclone
11 May 2014	9:00-10:00	Fair	1016.3	22.7	51	ESE	3.02	13	0.47	Anticyclone
22 May 2014	9:26-10:26	Cloudy	1009.0	20.6	74	SSE	1.07	4.9	1.8	Anticyclone
22 May 2014	12:00-13:00	Cloudy	1009.2	20.8	73	SSE	1.30	1.7	0.60	Anticyclone
22 May 2014	15:00-16:00	Cloudy	1009.1	20.7	75	E	0.82	2.2	0.56	Anticyclone
22 May 2014	18:00-19:00	Cloudy	1009.3	19.8	82	NNW	0.92	3.0	0.63	Anticyclone
22 May 2014	21:00-22:01	Fair	1011.4	16.3	92	N	0.68	7.8	0.80	Anticyclone
23 May 2014	0:00-1:00	Fair	1012.0	15.9	93	N	0.72	1.3	0.08	Anticyclone
23 May 2014	3:00-4:06	Fair	1012.3	15.4	92	N	0.57	1.6	0.21	Anticyclone
23 May 2014	6:00-7:00	Fair	1013.8	16.8	88	NNE	0.72	5.2	0.50	Anticyclone
23 May 2014	9:00-10:00	Fair	1014.6	22.8	60	S	0.87	9.3	0.79	Anticyclone

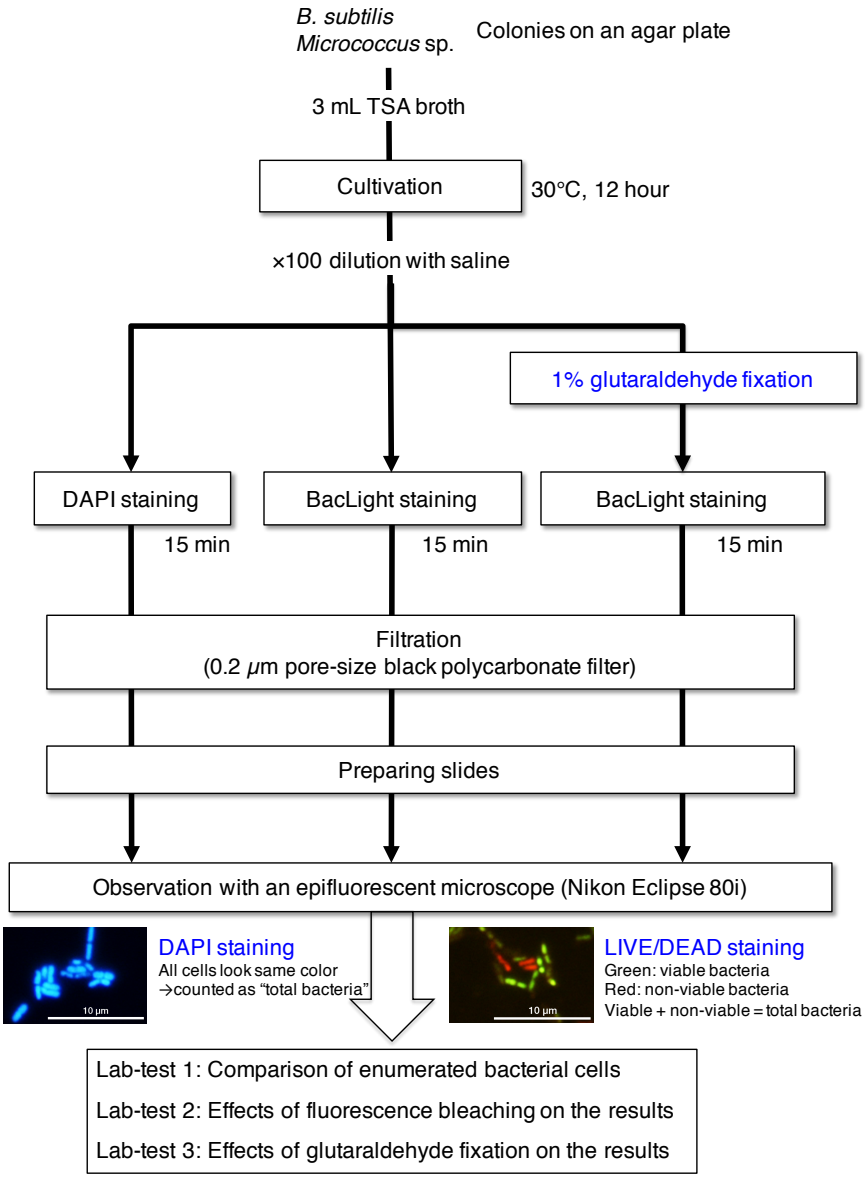

Figure S1. Flow chart of lab-tests for the BacLight stain.

Figure S2. Building for sampling at the Kumamoto site.

Fig S3. The look of a BioSampler.

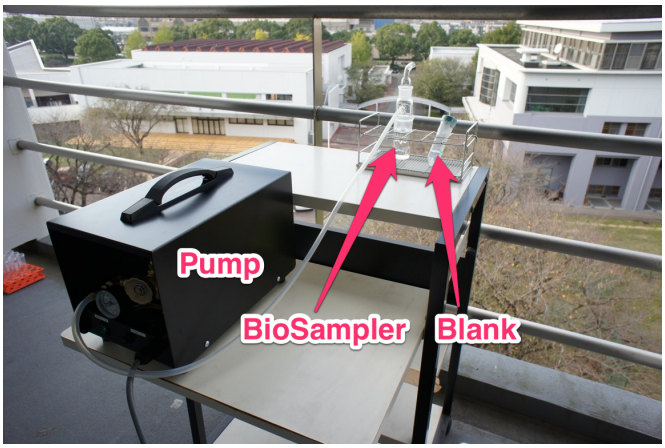

Figure S4. A picture showing what the sample collection looks like. Air samples were vacuumed into the BioSampler by the pump with the flow rate of 12.5 L min^{-1}. PBS media without exposing the air was prepared for a blank control and placed by the sampler during the collection.

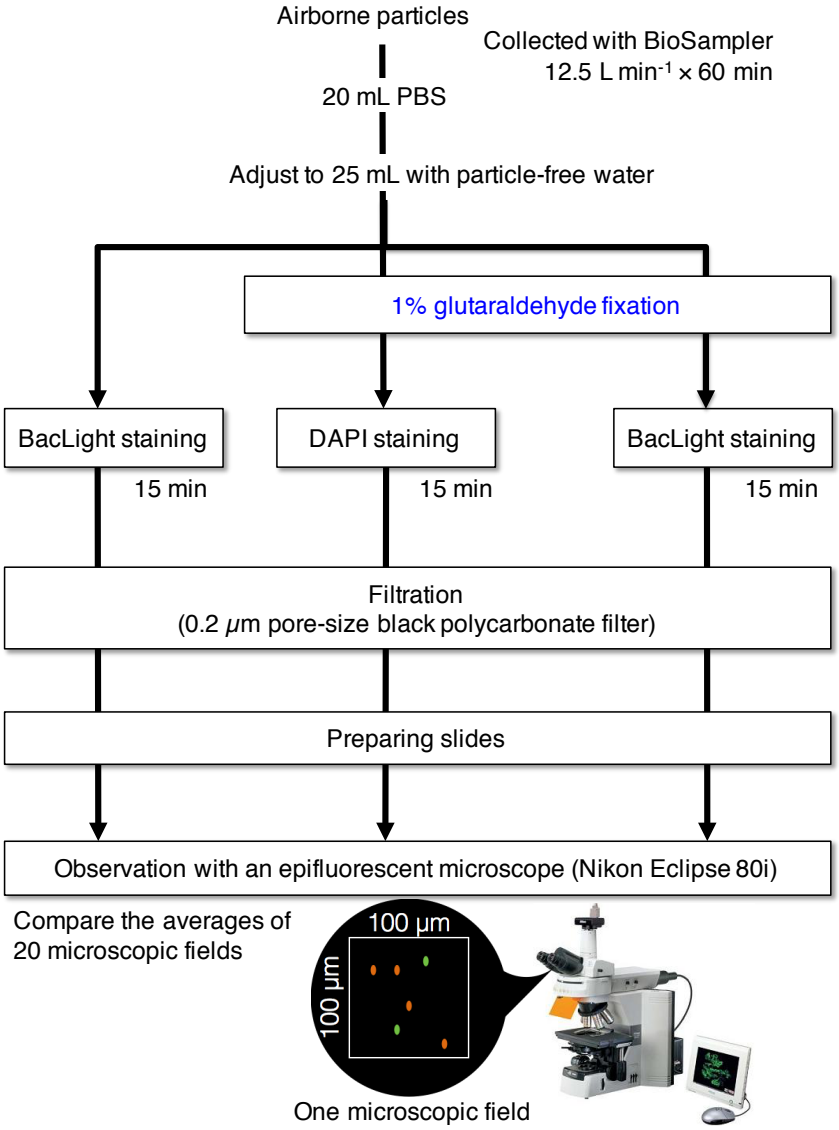

Figure S5. Flow chart of field-test of the BacLight stain.

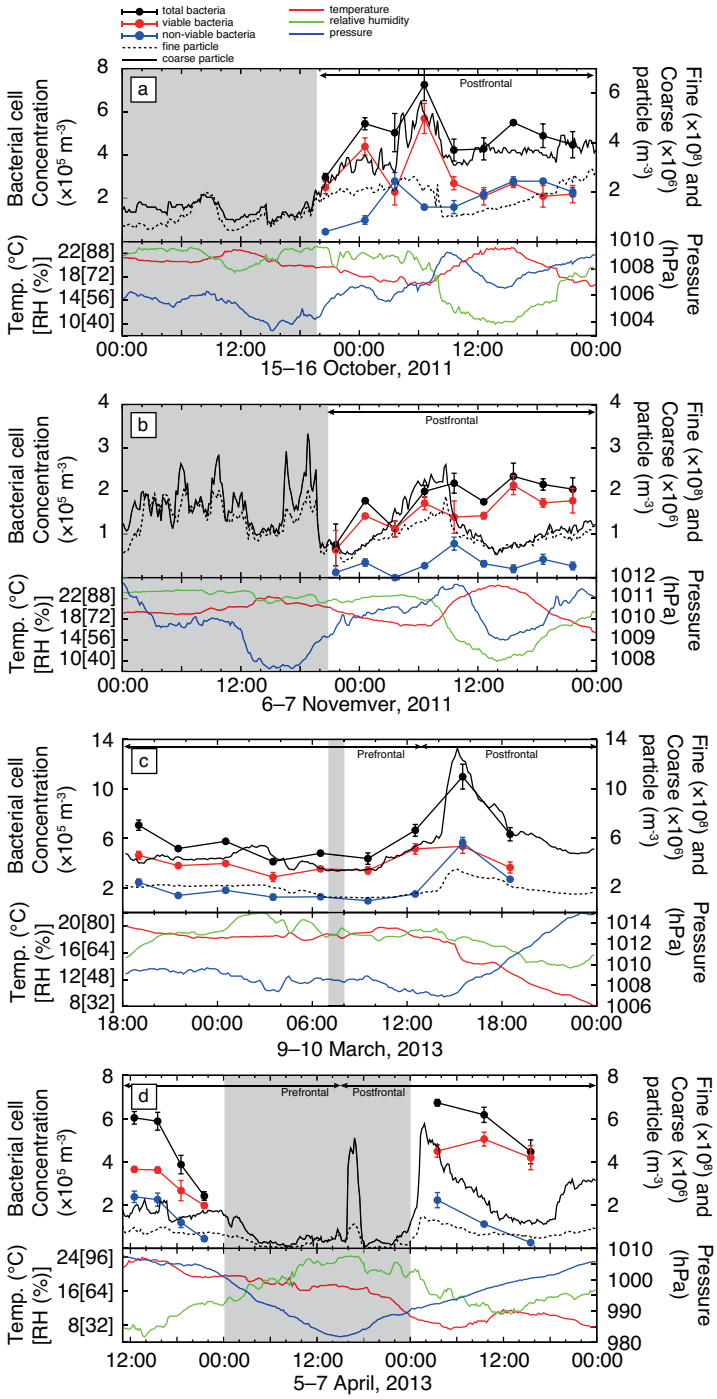

Figure S6. Viable, non-viable, and total bacterial concentration in the air at the Kumamoto site: (a) series 1, (b) 2, (c) 4 and (d) 5. Fine and coarse particle concentrations are shown as the dotted and solid lines, respectively. Temperature, relative humidity, and pressure also shown. (reprinted from *Murata and Zhang* [2013])

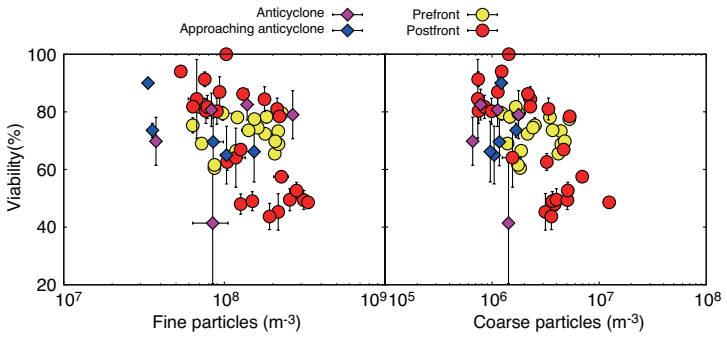

Figure S7. Bacterial viability vs. fine and coarse particles from the result obtained at the Kumamoto site. (reprinted from *Murata and Zhang* [2013])

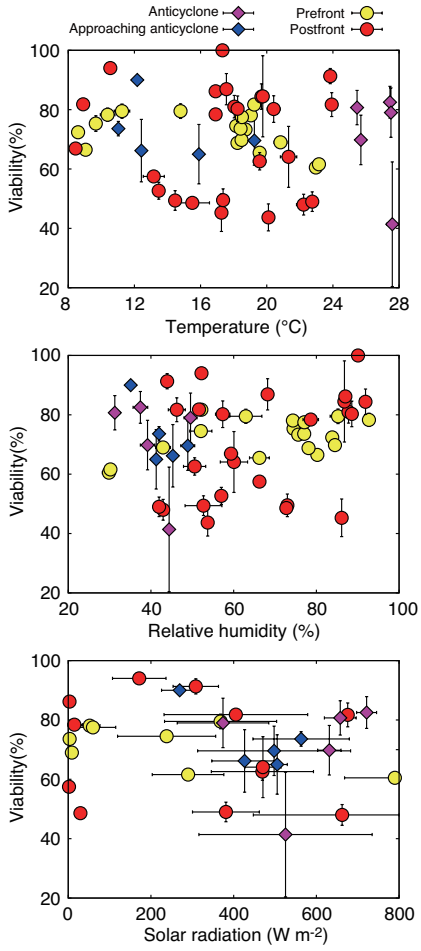

Figure S8. Bacterial viabilities vs. the temperature, relative humidity and solar radiation at sample collection at the Kumamoto site. Error bars for prefrontal and postfrontal viability mark the standard deviations in three replicated counts of each sample and those of anticyclones and approaching anticyclones were estimated from 20-microscopic fields. (reprinted from *Murata and Zhang* [2013])

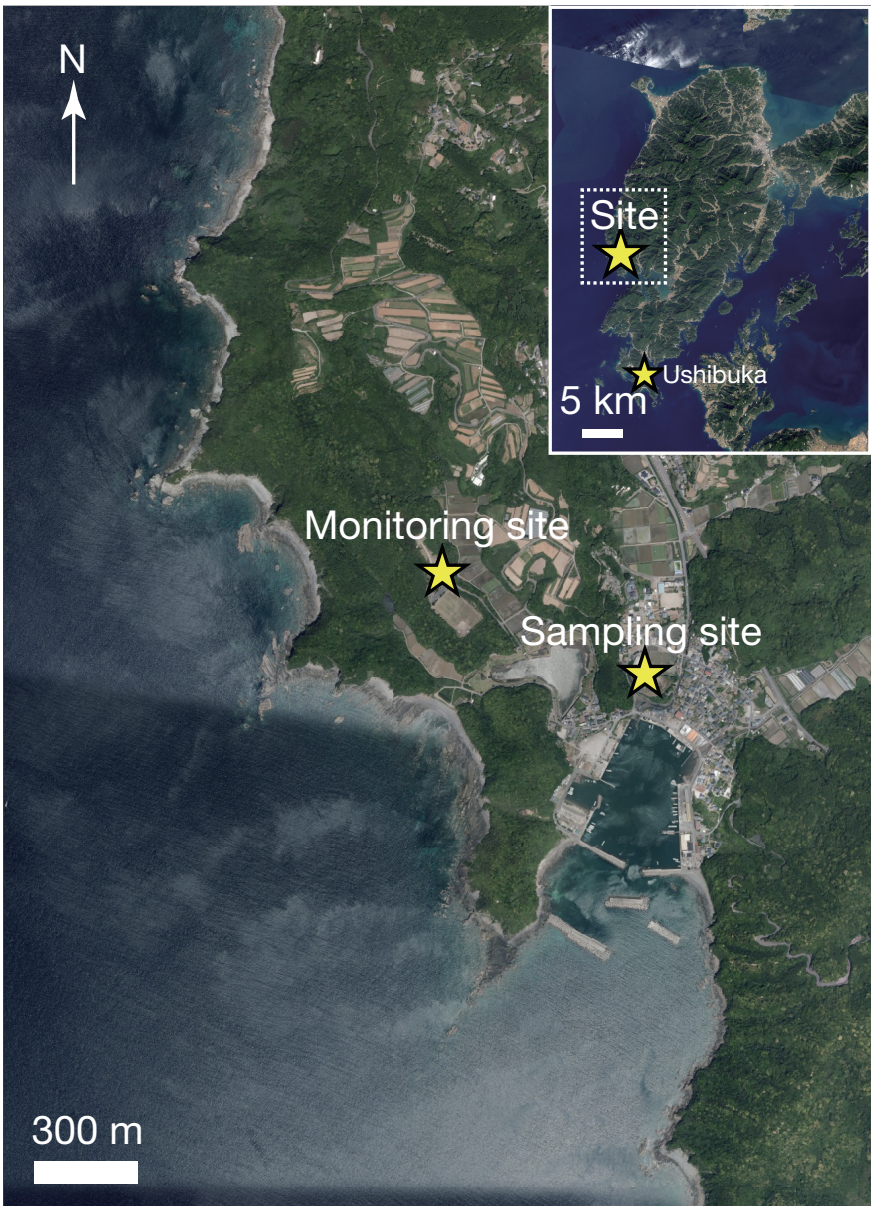

Figure S9. Geography of the sites in Amakusa. Meteorological data were measured at the monitoring site. The meteorological observatory we used to compensate the data losses is also shown (Ushibuka). The map was modified from the web map from Geospatial Information Authority of Japan (http://maps.gsi.go.jp/). (reprinted from *Murata and Zhang* [2016, *in submission*])

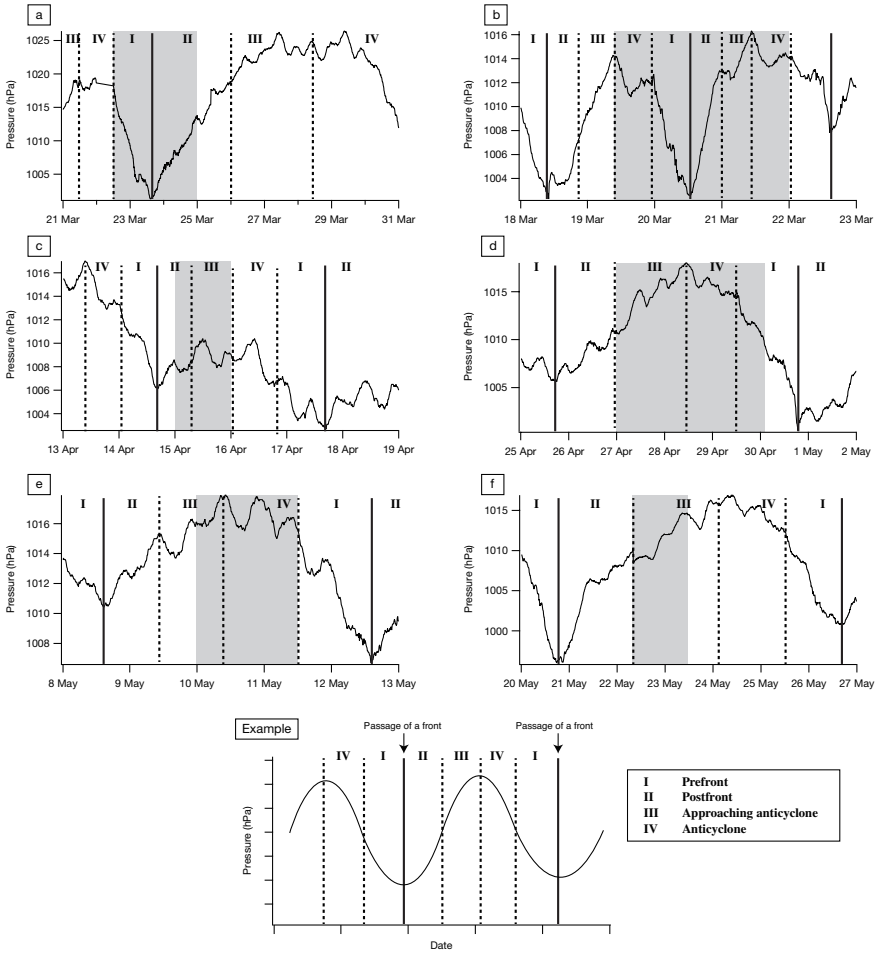

Figure S10. Classification of air parcels according to pressure variation for the results from the Amakusa site. The example shows a simplified illustration of pressure change during several days and how we classified the periods. The results of classification for observation periods are show as a: 22–24 March 2012, b: 19–21 March 2013, c: 15 April 2013, d: 27–29 April 2013, e: 10–11 May 2014, and 22–23 May 2014. The gray shades in the figure represent observation periods. (reprinted from *Murata and Zhang* [2016, *in submission*])

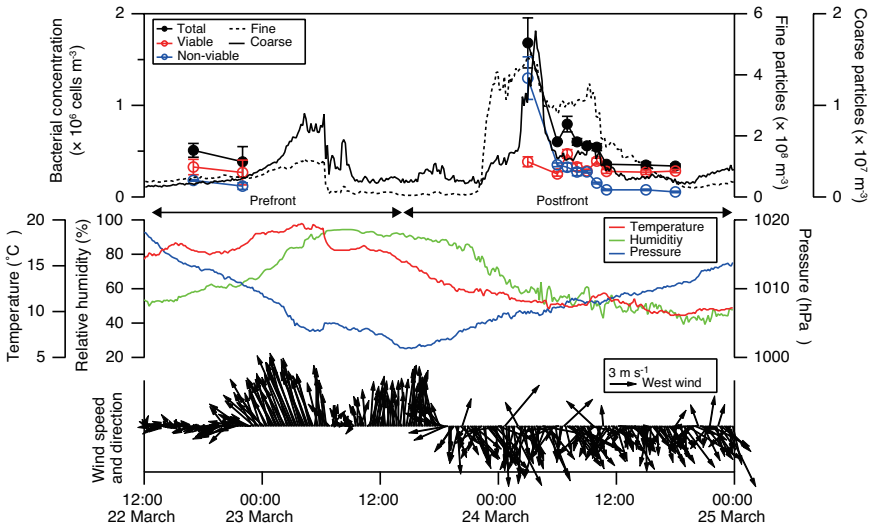

Figure S11. Evolution of the concentrations of airborne bacteria and fine and coarse particles at the Amakusa site from 12:00 on 22 March 2012 to 0:00 on 25 March 2012. Temperature, relative humidity, pressure and wind vectors are also shown. (created based on the data from *Murata and Zhang* [2016, *in submission*])

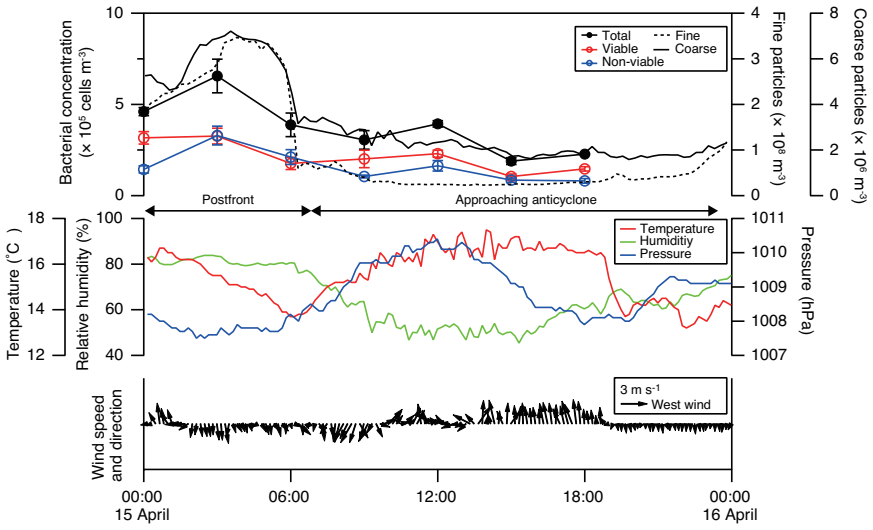

Figure S12. Same as in Figure S11 but for the period from 15 April 2013 to 16 April 2013. (created based on the data from *Murata and Zhang* [2016, *in submission*])

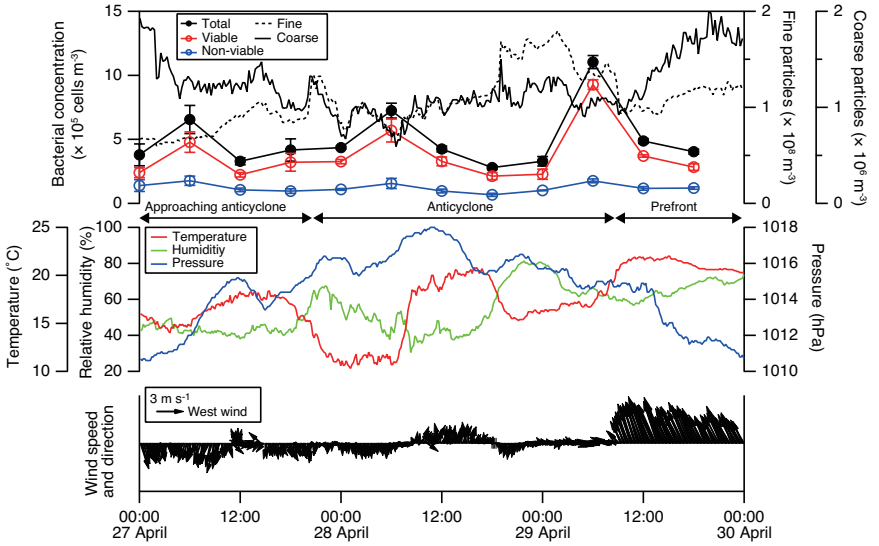

Figure S13. Same as in Figure S11 but for the period from 27 April 2013 to 30 April 2013. (created based on the data from *Murata and Zhang* [2016, *in submission*])

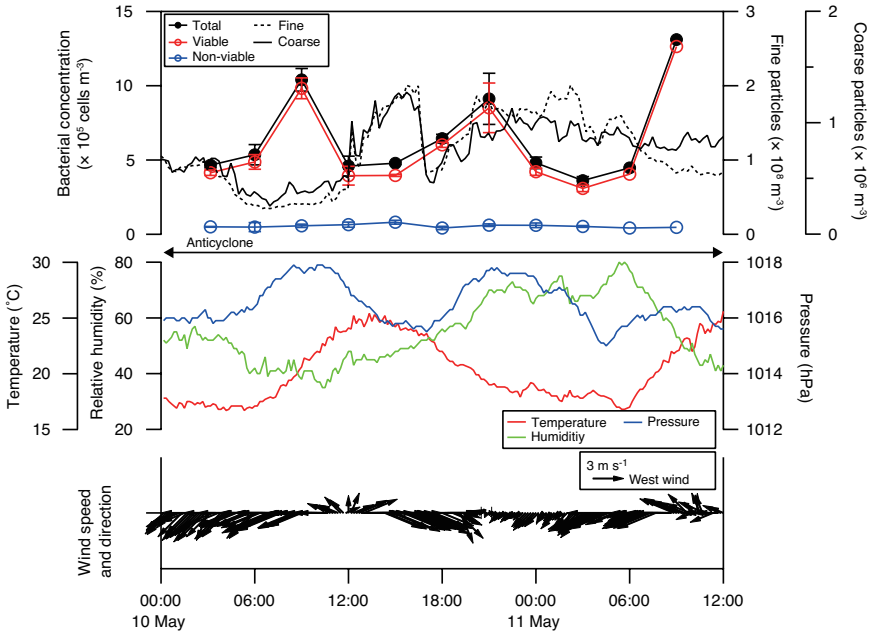

Figure S14. Same as Figure S11 but for the period from 10 May 2014 to 11 May 2014. (created based on the data from *Murata and Zhang* [2016, *in submission*])

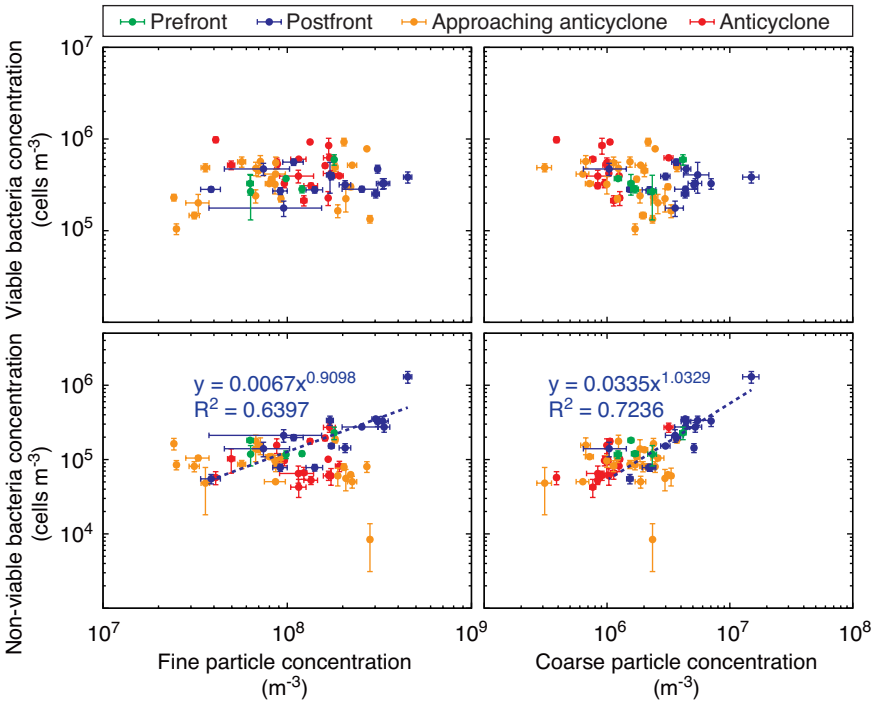

Figure S15. Viable/non-viable bacteria vs. fine/coarse particles under different weather conditions at the Amakusa site. Error bars indicate the standard deviations of three replicated counts of bacteria and of four 15 minutes-averaged counts of particles. (reprinted from *Murata and Zhang* [2016, *in submission*])

Supporting information: the pattern of land-sea breeze circulations in Amakusa

To show general diurnal wind pattern at the site in Amakusa under the anticyclonic conditions, we depicted wind direction and speed and the frequency of wind direction in three hours in March 2014 and 2015 (i and ii) and April 2013, 2014, and 2015 (iii and iv) and May 2012, 2013, and 2015 (v and vi) in Figure S16. The data were classified in the same manner described in Figure S10. The data in May 2013 and March in 2014 were not used because of a lot of data losses due to instrument errors. The wind at Amakusa was generally dominated by northerly, and diurnal wind shift was observed in May but the shift was weak in March and April. Wind in May was weak, indicating that the weather condition during anticyclone periods in May usually resulted in stagnant conditions, compared with those in March and April.

The wind in May was usually dominated by weak northerly winds. However, southerly sea breezes occurred from 9 am to 6 pm (vi), and the wind velocity was slightly higher than the northerly wind (v). The northerly wind returned at about 6 pm, and the wind velocity was usually lower than 1.5 m s^{-1}. On the other hand, the wind patterns in March and April was dominated by northerly and northeasterly flow. However, wind shift from northerly to northeasterly/easterly frequently occurred in the early morning at around 6 am. Southerly breeze was developed at 9 am and/or by 12 am at the latest, although the relative frequency was low. These diurnal changes in wind direction in spring indicate that land-sea breeze circulations generally occur at the site, although the pattern slightly differ in different months.

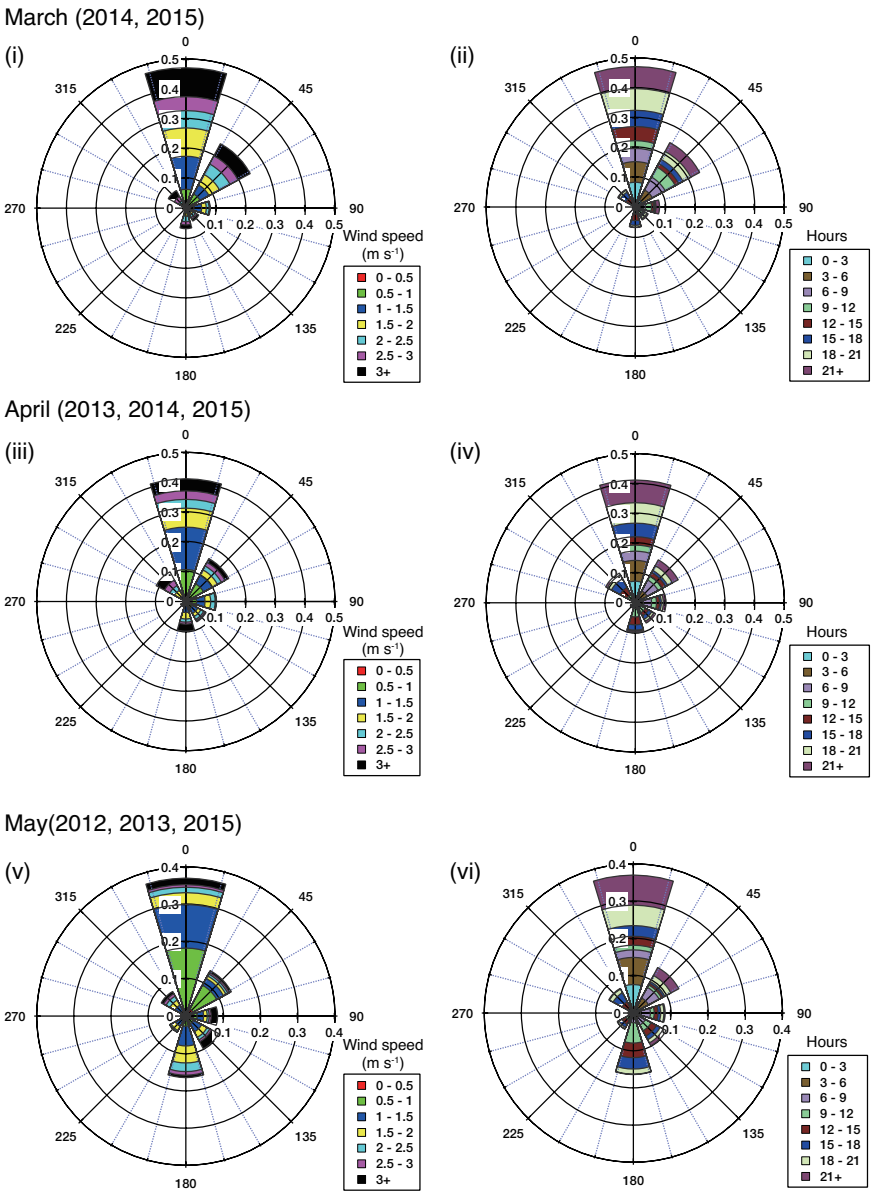

Figure S16. Diurnal wind patterns at the Amakusa site during approaching anticyclone and anticyclone periods in the springs of 2012–2015. The frequencies of wind direction with different wind speed in March, April, and May were depicted in (i), (iii), and (v), respectively. The frequencies of wind direction with different hours in the months were depicted in (ii), (iv), and (vi), respectively.