



# Air pollution and airborne infection with mycobacterial bioaerosols: a potential attribution of soot

J. Noda<sup>1</sup> · S. Tomizawa<sup>1</sup> · K. Takahashi<sup>1</sup> · K. Morimoto<sup>2,3</sup> · S. Mitarai<sup>3</sup>

Received: 30 May 2020 / Revised: 10 January 2021 / Accepted: 28 January 2021  
© Islamic Azad University (IAU) 2021

## Abstract

Atmospheric pollutants are hypothesized to enhance the viability of airborne microbes by preventing them from degradation processes, thereby enhancing their atmospheric survival. In this study, *Mycobacterium smegmatis* is used as a model airborne bacteria, and different amounts of soot particles are employed as model air pollutants. The toxic effects of soot on aerosolized *M. smegmatis* are first evaluated and excluded by introducing them separately into a chamber, being sampled on a filter, and then cultured and counted. Secondly, the bacteria–soot mixture is exposed to UV with different durations and then cultured for bacterial viability evaluations. The results show that under UV exposure, the survival rates of the low-, medium-, and high-soot groups are 1.1 ( $\pm 0.8$ ) %, 70.9 ( $\pm 4.3$ ) %, and 61.0 ( $\pm 17.6$ ) %, respectively. This evidence significantly enhanced survival rates by soot at all UV exposures, though the combinations of UV exposure and soot amounts revealed a changing pattern of survival rates. The possible influence by direct and indirect effects of UV-damaging mechanisms is proposed. This study indicates the soot-induced survival rate enhancements of *M. smegmatis* under UV stress conditions, representing the possible relations between air pollution and the extended pathogenic viability and, therefore, increased airborne infection probability.

**Keywords** Airborne pathogen · Combustion-related pollutants · Non-tuberculosis mycobacterium · Ultraviolet stress

## Introduction

### Air quality and role of bioaerosols

To establish regulatory standard values, air quality-related environmental investigations have focused on the chemical compositions of gaseous and particulate matter (PM), such as SO<sub>x</sub>, NO<sub>x</sub>, ozone, CO, PM<sub>10</sub>, and PM<sub>2.5</sub>. Recently, the attribution of biological components for air quality research has gained more attention. Studies on earth system to indoor

air quality have recently increased the knowledge on biological aerosols (bioaerosols) to ensure health status at varying levels (Fröhlich-Nowoisky et al. 2016; Fujiyoshi et al. 2017; Ruiz-Gil et al. 2020). One reason for investigating the air quality is for safeguarding our health, and including bioaerosol fractions is a natural step. Also, some bioaerosols are pathogenic, causing infectious diseases, such as the recent pandemic event with coronavirus (SARS-CoV-2), norovirus, influenza, Ebola virus, tuberculosis (TB), and other pathogens transmitted via aerosols (Wang and Du 2020; Doremalen et al. 2020; Liu et al. 2020; Jones and Brosseau 2015). The livestock sector has substantial concerns regarding airborne viruses, such as foot-and-mouth disease (Colenutt et al. 2016; Christensen et al. 2011; Poonsuk et al. 2018), Q-fever (Gregory et al. 2019), *mycoplasma* (Kanci et al. 2017), and mycobacterium-related Johne's disease (Richardson et al. 2019). Notably, it is a significant concern for human and animal healthcare providers and medical professionals who are in close contact with such pathogenic bioaerosols. A formidable feature of pathogens is its ability to multiply within the host and then further infect other hosts, thus deteriorating health conditions of a larger number of a

Editorial responsibility: Mohamed F. Yassin.

✉ J. Noda  
jnoda@rakuno.ac.jp

<sup>1</sup> School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan

<sup>2</sup> Division of Clinical Research, Fukujiji Hospital, Japan Anti-Tuberculosis Association, Tokyo, Japan

<sup>3</sup> Department of Mycobacterium Reference and Research, Research Institute of Tuberculosis Japan Anti-Tuberculosis Association, Tokyo, Japan



population; this is known as a pandemic case, which is difficult to control. However, many toxic chemical substances have a more explicit dose–response relationship, reducing exposure to chemical substances and alleviating the risk of health problems.

## Bioaerosols and atmospheric science

Atmospheric science can contribute to increasing knowledge of airborne infection mechanisms and the dispersion of pathogens to cause some illnesses. Thanks to advancements in analytical techniques in molecular biology, increased sensitivity and lowered detection limits contribute to understanding the role of bioaerosols in the atmospheric environment. Some approaches combine knowledge of dust events and meteorological datasets to reveal the long-distance transport mechanisms of bioaerosols. Kellogg and Griffin (2006) reported that dust derived from the Sahara Desert reaching the downwind region of the Caribbean ocean increased the fungi infection on coral leaf. Another example is Kawasaki disease (KD), a well-documented inflammatory syndrome predominantly occurring in children, which was initially diagnosed by Japanese physician Dr. Kawasaki in the 1960s; however, the etiological agent or mechanisms of KD are not fully understood (Ramphul and Mejias 2018). Some attempts to relate KD to climate conditions, such as wind patterns, were made (Rodó et al. 2016; Manlhiot et al. 2018). Although the precise mechanism of KD is undetermined, a multidisciplinary approach could further explain the illness and introduce a new implementation step. Understanding the role of bioaerosol is still in its initial stage; however, the above approaches could provide answers to manage health-related problems globally.

## Air pollution and bioaerosols

In the case of SARS-CoV-2 pandemic situation, various public places, such as schools, concert halls, theaters, public transportation, and many other crowded spaces, are high-risk areas for infecting the population. Knowledge on how bioaerosol pathogens maintain viability in such ambient conditions is limited; therefore, we must avoid public places. This study investigates this problem by combining knowledge of pathogens and other PMs. A previous investigation showed the significance of other atmospheric components. The viability of airborne bacteria aerosolized with marine sediment dust was significantly higher than the desert dust (Noda et al. 2019). This investigation showed that the examined marine sediment dust contained 4.7 times higher volatile organic fraction than desert dust, a common air pollutant; thus, soot could act as supporting material for bacteria. Some materials function as a supporting role; these materials are known as fomites, an object or substance that can transmit infectious

organisms from one individual to another (Fracastoro 1961; Contini and Costabile 2020). These results indicate a possible coupling between bioaerosol and dust PM as fomites that contribute to maintaining the viability of bacteria. Thus, bioaerosol behavior, including the pathogenic ones, significantly depends on the type and abundance of PM as fomites.

A recent SARS-CoV-2 publication shows that the infected patients' mortality rate correlates with long-term exposure to PM<sub>2.5</sub> in large cities of the USA (Wu et al. 2020, preprint). The 2003 SARS-CoV-1 outbreak in China, with a moderate or high long-term air pollution index (API), showed higher fatality rates than in locations with low API areas (Cui et al. 2014). Some reports have indicated the linkage between air pollution and infectious diseases to mortality rates (Di et al. 2017a, b; Croft et al. 2020; Di et al. 2017a, b; Tsai et al. 2019; Contini and Costabile 2020). A previous study in Okayama, Japan, indicated that cases of non-TB mycobacteriosis (NTM) caused by *Mycobacterium kansasii* had higher incidents with workers dealing with dust material in heavily industrial areas (Mimura 2002; Yoshida et al. 2011). Patients with NTM increased rapidly, exceeding the cases of TB since 2014 in Japan. The mechanism behind this trend is unclear (Namkoong et al. 2016), but there was a possible connection with metal dust material or air pollutants in the industrial area as fomites, mediating the transmission of pathogens. Furthermore, long-term exposure to high NO<sub>x</sub> levels could correlate with the SARS-CoV-2 fatality rate in European cities (Ogen 2020). These findings show that the level of air pollutants influences the fatality rates of those infections; however, the mechanisms are unclear. Furthermore, the host's immune response to the pathogen attributing to the actual infection must be considered; however, this is beyond the scope of this study.

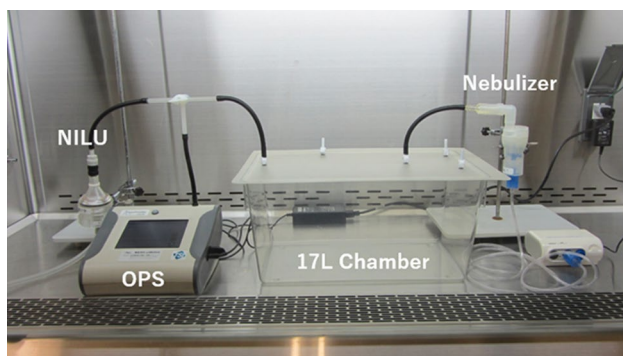
## Aim of the study

This investigation examined the hypothesis that soot can be a model air pollutant to maintain the viability of aerosolized mycobacterium under ultraviolet (UV) ray as a stress factor. Aerosolized bacteria and soot were collected on a membrane filter, and different durations of UV rays were irradiated as stress. Further culturing was conducted to evaluate the different viability of bacteria, and the survival rates of the bacteria were calculated. This study was carried out from 2018 to 2019 at Rakuno Gakuen University, Hokkaido, Japan.

## Materials and method

To examine the survival of bacterial bioaerosol, a simulation chamber system was used as a model of ambient air inside a safety cabinet (Fig. 1). It is designed to examine pathogenic





**Fig. 1** The polycarbonate chamber system (17 L) is attached with nebulizer, OPS, and the NILU filter unit

microorganisms; thus, the simulation chamber size was limited according to the safety cabinet size.

### Simulation chamber

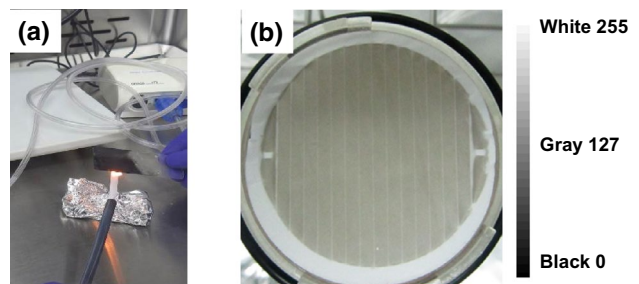
A simulation chamber made of a polycarbonate plastic cage was used in the examination (CLEA Japan Inc. Tokyo, Japan). Its dimensions are  $260 \times 425 \times 155 \text{ mm}^3$  with a volume of 17 L, and a lid made from a polypropylene sheet adhered to the cage with double-sided adhesive tapes, maintaining the tightness of the simulation chamber. Because the experiments were conducted inside the safety cabinet, the background air was high-efficiency particulate air (HEPA)-filtered particle-free air; thus, aerosolized bacteria and soot were diluted with particle-free air (Fig. 1).

### Nebulizer for aerosolization of bacteria

A jet nebulizer NE-C30 (OMRON Co. Kyoto, Japan) produces bioaerosols from a liquid bacterial solution using compressed air. The jet nozzle-type nebulizer is gentler than the ultrasonic type and is commonly used for respiratory tract humidification and inhalation therapy to administer drugs and other materials to human patients.

### Particle number size distribution

The concentration and size distribution of particles ranging from 0.3 to  $10 \mu\text{m}$  inside the simulation chamber before and after being introduced to the bacterial suspension or soot were determined using an Optical Particle Sizer (OPS; 3330, TSI Inc. Minnesota, USA), an online measurement device that directly monitors the concentration and size distribution of particles with a time resolution of a few seconds. Before the experiments, the ventilation mode of the safety cabinet was active, and inside the chamber, the system was purged with HEPA-filtered air for  $\sim 15$  min. After purging, the OPS verified that the inside of the chamber is particle free.



**Fig. 2** a Soot generation by incomplete combustion and b sampled filter to measure the amount of soot in gray scaling by ImageJ (the photograph shows the gray scale of 141)

### Model bioaerosols

This study used the bacterial strain (ATCC 19,420) *Mycobacterium smegmatis* (Jing et al. 2013; Louveau et al. 2005). Freshly cultured colonies were acquired from the Middlebrook 7H10 agar medium (Difco™, Maryland, USA) and were suspended in 1% bovine serum albumin (Roche, Basel, Switzerland) in phosphate buffer solution (Kent and Kubica 1985; Murray et al. 2007). Each fraction of serial dilutions was plated to determine a colony-forming unit (CFU) per volume *M. smegmatis* concentration. Before the experiment, bacterial concentrations were visually adjusted by comparing it with a standard turbidity solution (McFarland No. 3) with ca.  $9.0 \times 10^8$  CFU/ml. The adjusted bacterial suspension was kept at  $-20^\circ\text{C}$  until the ca. 1 h before nebulization. Modified Middlebrook 7H9 broth medium (Difco™, Maryland, USA) with oleic acid, albumin, dextrose, and catalase enrichment mixture was used for initial culturing with the filter sample and was plated and cultured with the 7H10 medium for CFU evaluation.

### Soot concentration

Soot, which was generated by burning a candle with incomplete combustion as the model soot, was used as a potential fomite in this experiment (Fig. 2a). After sampling the bacteria and soot mixture on the filter, pictures of sample filters were taken (Fig. 2b). The amount of soot on the filter was evaluated with 255 stages of gray scale produced by ImageJ software (Rasband 2018; Schneider et al. 2012). After editing the photographs to 32-bit monochrome, it was measured to determine the soot concentration on the filter using the ImageJ index.

### Sample filter and filter holder

A 47-mm-diameter polycarbonate filter holder, developed by the Norwegian Institute for air research (NILU, Oslo, Norway), was used in this investigation. A  $0.45\text{-}\mu\text{m}$ -pore size



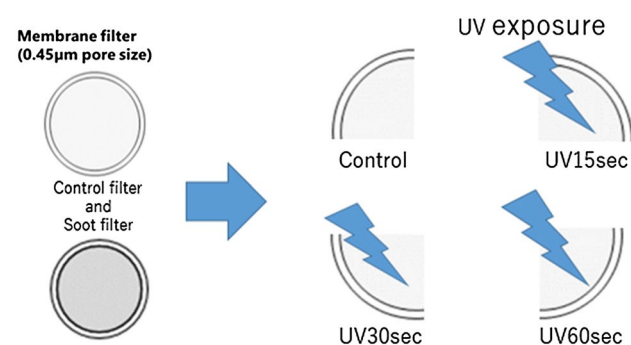
membrane filter made by polytetrafluoroethylene filter was used (Omnipore™, Merck KGaA, Darmstadt, Germany). The nebulized bacterial bioaerosol and soot in the chamber were collected on the filter at a flow rate of 9 L/min.

### Preparation of sample filters and culturing step

To prepare for the sample filters with only bioaerosol, the bacterial solution was first nebulized in the chamber for 3 min, with a 3-min waiting period for stabilization. Upon generating soot aerosols, it was suctioned into the chamber and collected on the filter inside the NILU filter holder. With a rate of 9 L/min air suction, 27-L air mass was sampled. A linear motor-free piston-type pump VP0940 (Nitto Kohki, Tokyo, Japan) was used with a digital mass flow controller MQV0050 (Azbil Co., Tokyo, Japan) to maintain the flow. For the preparation of a sample filter containing bioaerosol and soot, the soot generated from the candle was simultaneously introduced into the chamber to withdraw the pre-installed bioaerosol. After sample collection, the filter was removed from the NILU filter holder. Each sample filter was divided into four equal parts using sterilized scissors. Figure 3 shows that a quarter of the divided filter was used as a control, and the remaining three quarters were irradiated with UV for 15, 30, and 60 s each, respectively. Directly after the UV irradiation step, each sample filter was placed into separate 1.5-ml microtubes containing 1 ml of 7H9 broth medium, was mixed well with a vortex mixer, and incubated at 37 °C for 24 h. After the incubation period, each filter aliquot of 200 µl was serially diluted and cultured on 7H10 media plates for 96 h to calculate CFU.

### Ultraviolet irradiation

A germicidal lamp GL-15 (Panasonic, Osaka, Japan) in a safety cabinet was used as the UV ray source with 254 nm peak intensity. The exposed power of the UV rays was set to 60 µW/cm<sup>2</sup> by adjusting the distance to the filter surface



**Fig. 3** Either bacteria only or bacteria with soot were divided into four fractions with UV exposures of 0, 15, 30, and 60 s

from the lamp and exposure duration of 15, 30, and 60 s to simulate environmental stresses. This UVC intensity was monitored using the UV ray meter UVC-254 (CUSTOM, Tokyo, Japan).

### Statistical analysis

Data are summarized as average values with the standard error of the mean (SEM). Students' *t* tests were used to statistically calculate significant differences. *p* < 0.05 was considered statistically significant.

### Calculation of survival rate

The survival rate of *M. smegmatis* was estimated using the CFU counts and compared with a set of experiments without soot as a control bioaerosol sample (Eq. (1)):

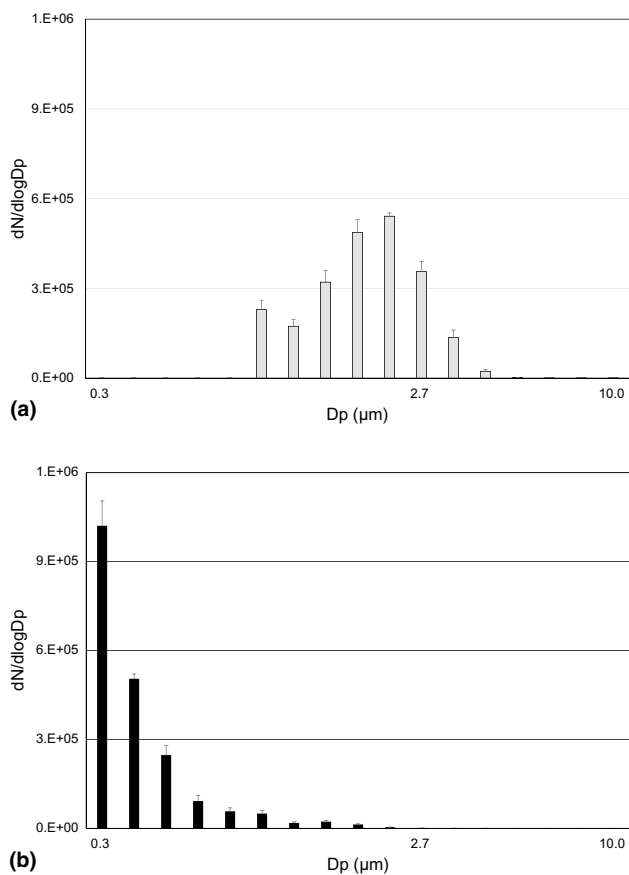
$$\text{Survival rate (\%)} = \frac{\text{CFU with soot}}{\text{CFU without soot}} \times 100. \quad (1)$$

## Results and discussion

### Optical particle sizer (OPS) result

The measurement data of particle concentrations and their size distributions calculated by OPS are shown in Fig. 4 (a) with nebulized *M. smegmatis* bacteria and (b) with generated soot. From the triplicated measurements, the average value and standard errors ( $1 \pm \text{SEM}$ ) are calculated and shown on the error bar. The nebulized bacteria in Fig. 4a show a peak concentration around 2 µm, which is an expected aerodynamic diameter range because *M. smegmatis* has a rod shape of 2–5 µm in length and a diameter of less than 1 µm (Kang et al. 2012). The freshly generated soot result shows 0.3 µm as the highest concentration in this measurement range. However, the soot generated from candle burning shows 50–100 nm size ranges as maximum concentrations (Pagels et al. 2009; Mulay et al. 2019). The real peak concentration could occur in a much smaller size range. Thus, Fig. 4b shows the tail part of soot particles, and the soot particle concentration increases as the size decreases; therefore, it is plausible to have a 50–100 nm size range as the peak concentration. Because the OPS uses a laser beam as a light source, there is a limitation in measuring particle sizes smaller than 0.3 µm in aerodynamic diameter. Reconfirming with the scanning mobility particle sizer or other nanoparticle monitoring devices is necessary. Monitoring aerosols using OPS confirms the introduction of bacteria and soot aerosols; thus, the measurement results served their purpose. For a more detailed contribution of nanosized range particles for the survival of bacteria, they must also be monitored.





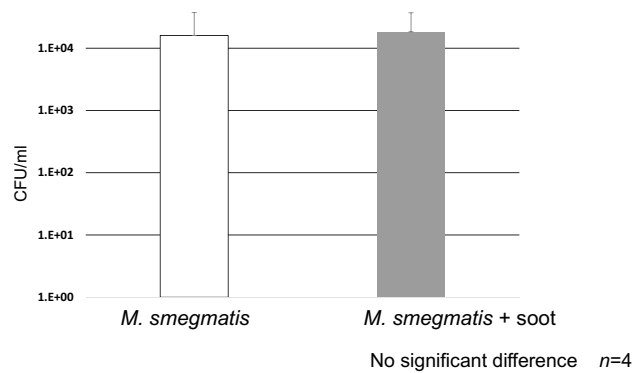
**Fig. 4** **a** Distribution of particle size and particle number of bioaerosol generated by nebulization of bacterial fluid. **b** Distribution of soot particle size and particle number after introducing soot into a 17-L chamber for 1 min

### Soot amount estimation on the filter

Figure 2b shows a filter that collected bacteria and soot with a gray scale of 141. The filter could have an uneven coloring with some lines because of the grid-type filter support placed under it. A large part of the filter area was photographed and analyzed for ImageJ as an average value considering the unevenness of soot distribution. Also, it was used only for estimating the soot accumulated on the filter. The average value and standard errors ( $1 \pm \text{SEM}$ ) of the obtained low, medium, and high soot conditions were 163 ( $\pm 8$ ), 86 ( $\pm 3$ ), and 12 ( $\pm 5$ ), respectively. The high soot condition had the highest SEM value relative to the average value. The darker the color, the harder it becomes to judge by the naked eye, and to determine the amount of soot, a precise measurement method is necessary.

### Culture result in the presence of soot

As an initial step, the toxic effect of soot on bacteria was examined. The filter samples of aerosolized bacteria and



**Fig. 5** The growth of aerosolized *M. smegmatis* only (left) and *M. smegmatis* with soot (right) showed no significant difference ( $p > 0.05$ )

the mixture of aerosolized bacteria and soot were cultured for CFU determination. Figure 5 shows the results from repeated trials ( $n = 4$ ) for bacteria only or bacteria with soot groups, showing that there was no significant difference in CFU ( $p > 0.05$ ). Because soot contains toxic substances, we anticipated less CFU for the group mixed with soot. Soot contains polyaromatic hydrocarbons (PAHs), which could have genotoxicity, and coexisting bacteria with soot could result in less CFU. Some PAHs have a strong carcinogenic effect on mammals. For example, benzo-a-pyrene has genotoxicity to inhibit bacterial growth (Samburova et al. 2017). The results from culturing bacteria with soot did not show a toxic effect from the reduced CFU. However, it was only the result of a culture-based test for ~96 h that showed that unnoticed damage to bacterial DNA might have occurred. Further serially passaged culturing could reveal genetic damage to the bacteria. Genetic-level damage can be further analyzed by the emergence of DNA repair proteins induced by the *recA* gene, a genetic marker for this process (Vollmer et al 1997; Rosen et al. 2000). Because the toxic effect of PAH to bacterial DNA was recognized, detecting the *recA* gene is a more direct evidence to show the bacterial response from soot exposure. Although the amount of PAH and other pollutants in the soot from the paraffin-type candles can widely vary (Derudi et al. 2014), the evaluation step requires further consideration of a soot property analysis. A detailed evaluation of genetic-level response to soot exposure is beyond the scope of this investigation; however, it is a direct way to assess the effect of soot on bioaerosol.

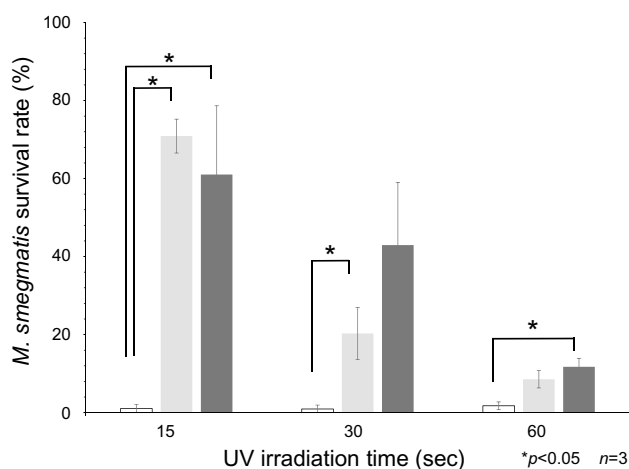
### Culture method results with UV ray irradiation as a stress factor

The sampled aerosolized bacteria and soot were first collected on the filter and then irradiated with UV to understand the effect of the soot on bacterial survival under the UV ray stress condition. This sampled filter was divided into

four parts, and each quarter was irradiated with the UV ray for 15, 30, and 60 s. Figure 6 shows the average survival rates ( $n=3$ ), and the error bars represent  $1 \pm \text{SEM}$  for low, medium, and high soot groups, with average gray scales of 163, 86, and 12, respectively. With the 15-s UV exposure test, the survival rate of *M. smegmatis* with the low soot group showed a 1.1% ( $\pm 0.8$ ) survival rate; however, the medium and high soot groups showed a significantly higher survival rate of 70.9% ( $\pm 4.3$ ) and 61.0% ( $\pm 17.6$ ), respectively ( $p < 0.05$ ). Similarly, the 30-s UV exposure test showed that the medium soot group displayed a significantly higher survival rate of 20.3% ( $\pm 6.7$ ) than the low soot sample group with 0.9% ( $\pm 0.8$ ) ( $p < 0.05$ ). Lastly, the 60-s UV exposure test showed a significantly higher survival rate of 11.7% ( $\pm 2.2$ ) in the high soot group than the low soot group with 1.8% ( $\pm 1.8$ ) ( $p < 0.05$ ). Notably, the 30- and 60-s UV exposure experiments showed a higher survival rate with the high soot groups.

The series of experiments showed that soot helps survive UV exposure. However, the more considerable amount of soot does not contribute to the survival of the bacteria. Thus, more sophisticated mechanisms are needed to derive the above results. Figure 6 shows that the survival rate for medium and high soot groups decreased with extended UV irradiation time from 15 to 60 s. The survival rates of the medium soot group decreased more rapidly than the high soot groups as UV irradiation time was extended.

Interestingly, the survival rates between medium and high soot groups were inverted after 30 s. The higher soot group survived longer than the medium group for 30 and 60 s, which could be because of a different mode of bacterial damage. Nelson et al. (2018) reported different microorganism damaging modes with different UV wavelengths showing



**Fig. 6** The average survival rates affected by soot on *M. smegmatis* against UV irradiation of 15, 30, and 60 s. The gray color bars show the different amounts of soot; low with the white, medium with the gray, and high with the black at each UV irradiation period

more dominant endogenous UV effects (direct one) for UVB and more dominant exogenous UV effects (indirect) for UVA. In this investigation, the medium soot condition allows a certain proportion of UV rays to directly reach the bacteria, causing damage to decrease the survival rate. With the high soot condition, the soot absorbs most of the UV rays, hindering the direct damage to the bacteria. However, the absorption of UV rays on soot could generate a photo-produced reactive intermediate (PPRI) that could predominantly have an indirect toxic effect, reducing the survival rate of bioaerosols. The mechanism proposed by Nelson et al. (2018), UV, and some visible rays could produce PPRI to have an exogenous indirect effect on the microorganisms.

The UV absorption on soot could produce PPRI, indirectly damaging the microorganisms. Our investigation only used UVC rays to examine the protective features of soot, and the extent of PPRI generation using UVC rays was not investigated. Also, the toxicity of the indirect exogenous UV effect highly depends on the chemical properties of the soot, which could pose more direct differences for the survival rates of bacteria. Having a combination of direct and indirect effects to reduce survival rates is possible; however, further verification is necessary to quantify the produced PPRI under different UV exposure conditions. One approach is determining the reactive oxidant species (ROS), one of the PPRI, using the fluorescence technique (McBee et al. 2017). We anticipate ROS to increase with longer UV exposure time to the soot. Shiraiwa et al. (2012) reported that soot and polycyclic aromatic compounds could trigger ROS formation; however, several mechanisms exist to initiate ROS formation. UV irradiation should be considered one of many reaction steps in atmospheric conditions.

## UV wavelength

In this experiment, we only use the UVC wavelength, which does not reach ground level. Further studies on irradiation using UVB and UVA could provide results with realistic circumstances at the near-ground atmospheric condition. A different wavelength of UVB to visible light has a different inactivation mechanism for specific microorganisms with different mechanisms (Nelson et al. 2018). The endogenous and exogenous effects can damage the virus and bacteria with mostly UVB, and the endogenous indirect effect can damage bacteria with mostly UVA. Because different UV wavelengths could have different mechanisms and roles to cause damage to bacteria, further investigation is critical. Also, the chemical composition of the bacterial outer layer could vary with each species; the type and amount of PPRI might vary accordingly.

In this investigation, we examined *M. smegmatis*, which has an outer layer covered predominantly with mycolic acid, a group of long fatty acids that have chemical composition

that varies under varying conditions (Verschoor et al. 2012). The complex entities of mycolic acid have different potentials to generate PPRI of different wavelengths; however, this requires further investigation. Besides, the soot attachment and distribution patterns on the bacterial surface could have a different hindering effect on the UV ray to penetrate through the bacteria. Soot absorbs the UV ray to protect bacteria because soot has a relatively high absorption as the wavelength reaches the UV ray range (Russo et al. 2017). However, the location of the soot attachment provides different protective effects. The soot coverage in the most susceptible area has a more significant contribution to the survival of bacteria. The coating pattern of soot is significant for the UV ray to reach bacteria to have both direct and indirect effects. UV wavelength-dependent experiments are necessary to evaluate the different modes of damage to bacteria.

### Soot and bacterial interaction

An outer layer of bacteria consists of materials such as mycolic acid, trehalose complex, polysaccharides from the biofilm, and other substances. The attachment of soot to the outer layer of bacteria could have specific selectivity because of affinities determined by charge, chemical, physical, and other properties. Under the limited soot condition, the soot attaches to the surface material with the most definite affinity, causing uneven distribution. However, in an abundant soot condition, soot might attach to an even less avid position with a weak affinity, making the attachment pattern more random and complicated. With the excess soot condition, multilayers of soot can form on the surface of bacteria, including unique conformation patterns to protect or damage the bacteria. Also, the high soot condition becomes more susceptible to environmental changes or experimental conditions, such as temperature, humidity, and static electricity. A more considerable amount of soot condition allows different mechanisms, resulting in more variation on the survival rates to cause greater SEM values. Fewer of the  $1 \pm \text{SEM}$  values were observed for the medium soot group than the high soot group, except for the 60 s of UV irradiation. The limited soot condition of the medium soot group has a more uniform soot distribution; therefore, the effect of UV irradiation protects and damages the bacteria similarly. The excess soot condition applies to the high soot group where soot attachment becomes more random and complex, causing a substantial variation in the SEM values.

*Mycobacterium smegmatis* contains mycolic acid, a long carbon chain with many lipophilic layers that could have a high avidity with soot; however, the bacteria can adapt to a specific environment that could change the outer layer composition, producing different characteristics and affinity (Verschoor et al. 2012; Li et al. 2012). Thus, varying affinity to soot causes a more prominent variability in the

survival rates. Another study on the biological response on the presence of black carbon showed drastic changes in the development of bacterial biofilm, which is critical for bacterial colonization and survival (Hussey et al. 2017). Hussey et al. (2017) proposed that the presence of soot could send a signal to bacteria for biofilm formation enhancement with *Staphylococcus aureus* and *Staphylococcus pneumoniae* to resist certain antibiotic drugs. It illustrates the potential mechanism of coexisting soot acting to send a chemical signal to trigger the bacteria to form some protective properties, indicating that bacteria being inhaled with or without soot could cause a more drug-resistant-type infection.

### Soot and infectious diseases

With the SARS-CoV-2 pandemic, the linkage between mortality and exposure to air pollution in the USA was reported as a nationwide cross-sectional long-term epidemiological study (Wu et al. 2020). Previous reports illustrate similar findings with increased levels of PM air pollution, which exacerbates respiratory infections (Croft et al. 2020; Horne et al. 2018). The outbreak of severe acute respiratory syndrome (SARS) in 2003, a similar type of coronavirus to SARS-CoV-2, in high API cities had higher fatality rates than locations with low API (Cui et al. 2003). Investigating the interaction between pathogens and air pollutants is essential. Many reports illustrate the linkage between air pollution and respiratory infections. The challenge is that the immune response on the host side could also have a significant influence, and the immune response is complex and has substantial individual differences. Understanding the linkage between the frequency of infection and the level of air pollutants is challenging. One might need to consider individual monitoring or small-scale measurements to understand the link.

Subsequently, understanding the mechanism of how the pathogen maintains infectivity for a prolonged period in the real atmospheric environment under different stresses such as temperature, humidity, UV, and atmospheric oxidant species is critical. There are numerous studies on fomite surfaces such as a doorknob, medical instruments, and other surfaces with the hand contact area as a source of infection; however, the potential of airborne fomites, such as PM, has not received much attention. Our previous attempt to investigate the mixture of bioaerosol and different micron- to submicron-size PM showed a significant difference in survival rate (Noda et al. 2019). The bacteria with PM from marine sludge had significantly higher survival rates than those from the desert sand origin. The marine sludge had almost five times higher total organic content than desert sand, indicating that soot could have a common chemical feature with high carbon content. This investigation used soot as the organic components of air pollutants



to understand the contributing factors for bacteria to maintain their viability, which we humans want to counteract to reduce the risk of infection. The *M. smegmatis* used in this study is UV irradiation-sensitive; hence, soot is a potential protective fomite; however, other bacteria or viruses could have suitable fomites to evade specific stresses. Understanding the protective aspect of airborne fomites with different materials could reveal the potential risk of infection for different pathogens and reduce the risk of airborne infection by minimizing the presence of such fomites.

### Limitation and suggestions for further research

This work examined only UVC irradiation as a stress factor, which limits the extrapolation of the results to the real tropospheric environment. Since the tropospheric condition receives only UVA and UVB, investigating the irradiation of longer UV wavelengths is necessary to simulate a more realistic situation. In this investigation, the examined soot was freshly generated, and the oxidation of soot was low compared to the atmospheric soot suspended in the air and subject to the aging process. The aged soot with an oxidized form could become more reactive to pose varying toxicity to airborne bacteria; thus, the type and amount of oxidized products of soot need to be confirmed more precisely. UV irradiation could induce PPRI to damage the bacteria, and even the soot itself might undergo an oxidation process to generate the PPRI to damage the bacteria. One approach is stimulating the generation of PPRI by mixing the soot with ozone or other oxidants and exposing the bacteria for the toxic effect of soot. Furthermore, this investigation uses only the candle-generated soot as model soot. Other soot types from different fuel combustion could provide a more realistic condition.

For the bacterial response to stress, this investigation used only colony-forming ability as an indicator of damage or stress; however, bacteria could show stress response by direct genetic damage or damage response by the stimulated repair function of bacteria. Lastly, this investigation provides only a small part of soot and bioaerosol interaction and is in a preliminary stage.

### Conclusion

The experimental results indicated no direct inhibition of *M. smegmatis* growth with soot; thus, the soot did not have a direct toxic effect. However, this investigation examined the inhibition of *M. smegmatis* growth with freshly generated soot, and aged or more oxidized soot could provide different results. Also, it was only the result of a culture-based test for ~96 h, and unnoticed damage to bacterial DNA might have occurred.

Furthermore, serially passaged culturing or other genotoxicity tests could reveal genetic toxic damage to *M. smegmatis*. A further examination of UV stress conditions for the growth inhibition of aerosolized *M. smegmatis* and three types of soot collected on a filter was conducted. The results revealed that soot significantly contributed to an increase in the survival rate of *M. smegmatis*. However, the results from varying UV stress periods and soot amounts showed different survival rate changes. The possible involvement of direct and indirect UV-damaging mechanisms was suspected.

The freshly generated soot from the candle burning indicated a potential attribution for the increased survival rate of *M. smegmatis* under the UV stress condition. The result could explain the possible linkage between air pollution and the pathogens' extended viability to increase airborne infection probability.

**Acknowledgements** This work was supported by JSPS KAKENHI Grant Number JP15K00568 from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

### Compliance with ethical standards

**Conflicts of interest** The authors declare no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

### References

- Christensen LS, Brehm KE, Skov J, Harlow KW, Christensen J, Haas B (2011) Detection of foot-and-mouth disease virus in the breath of infected cattle using a hand-held device to collect aerosols. *J Virol Methods* 177(1):44–48. <https://doi.org/10.1016/j.jviro.2011.06.011>
- Colenutt C, Gonzales JL, Paton DJ, Gloster J, Nelson N, Sanders C (2016) Aerosol transmission of foot-and-mouth disease virus Asia-1 under experimental conditions. *Vet Microbiol* 189:39–45. <https://doi.org/10.1016/j.vetmic.2016.04.024>
- Contini D, Costabile F (2020) Does air pollution influence COVID-19 outbreaks? *Atmosphere* 11:377. <https://doi.org/10.3390/atmos11040377>
- Croft DP, Zhang W, Lin S (2020) Associations between source-specific particulate matter and respiratory infections in New York state adults. *Environ Sci Technol* 54(2):975–984. <https://doi.org/10.1021/acs.est.9b04295>
- Cui Y, Zhang ZF, Froines J, Zhao J, Wang H, Yu SZ, Detels R (2003) Air pollution and case fatality of SARS in the People's Republic of China: an ecologic study. *Environ Health* 2(1):15. <https://doi.org/10.1186/1476-069X-2-15>
- Derudi M, Gelosa S, Sliepcevich A, Cattaneo A, Cavallo D, Rota R, Nano G (2014) Emission of air pollutants from burning candles with different composition in indoor environments. *Environ Sci Pollut Res Int* 21(6):4320–4330
- Di Q, Dai L, Wang Y, Zanobetti A, Choirat C, Schwartz JD, Dominici F (2017a) Association of short-term exposure to air pollution with mortality in older adults. *JAMA* 318(24):2446–2456. <https://doi.org/10.1001/jama.2017.17923>





- Di Q, Wang Y, Zanobetti A, Wang Y, Koutrakis P, Choirat C, Dominici F, Schwartz J (2017b) Air pollution and mortality in the Medicare population. *N Engl J Med* 376(26):2513–2522. <https://doi.org/10.1056/NEJMoal702747>
- Fröhlich-Nowoisky J, Kampf CJ, Weber B, Huffman JA, Pöhlker C, Andreae MO, Lang-Yona N, Burrows SM, Gunthe SS, Elbert W, Su H, Hoor P, Thines E, Hoffmann T, Després VR, Pöschl U (2016) Bioaerosols in the earth system: climate, health, and ecosystem interactions. *Atmos Res* 182:346–347. <https://doi.org/10.1016/j.atmosres.2016.07.018>
- Fujiyoshi S, Tanaka D, Maruyama F (2017) Transmission of airborne bacteria across built environments and its measurement standards: a review. *Front Microbiol* 8:2336. <https://doi.org/10.3389/fmicb.2017.02336>
- Gregory AE, van Schaik EJ, Russell-Lodrigue KE, Fratzke AP, Samuel JE (2019) *Coxiella burnetii* intratracheal aerosol infection model in mice, guinea pigs, and nonhuman primates. *Infect Immun*. <https://doi.org/10.1128/IAI.00178-19>
- Horne BD, Joy EA, Hofmann MG (2018) Short-term elevation of fine particulate matter air pollution and acute lower respiratory infection. *Am J Respir Crit Care Med* 198(6):759–766. <https://doi.org/10.1164/rccm.201709-1883OC>
- Hussey SJK, Purves J, Allcock N, Fernandes VE, Monks PS, Ketley JM, Andrew PW, Morrissey JA (2017) Air pollution alters *Staphylococcus Aureus* and *Streptococcus Pneumoniae* biofilms, antibiotic tolerance and colonisation. *Environ Microbiol* 19(5):1868–1880. <https://doi.org/10.1111/1462-2920.13686>
- Jing W, Zhao W, Liu S, Li L, Tsai CT, Fan X, Wu W, Li J, Yang X, Sui G (2013) Microfluidic device for efficient airborne bacteria capture and enrichment. *Anal Chem*. 85(10):5255–5262. <https://doi.org/10.1021/ac400590c>
- Jones RM, Brosseau LM (2015) Aerosol transmission of infectious disease. *J Occup Environ Med* 57(5):501–508. <https://doi.org/10.1097/JOM.0000000000000448>
- Kanci A, Wawegama NK, Marenda MS, Mansell PD, Browning GF, Markham PF (2017) Reproduction of respiratory mycoplasmosis in calves by exposure to an aerosolized culture of *Mycoplasma bovis*. *Vet Microbiol* 210:167–173. <https://doi.org/10.1016/j.vetmic.2017.09.013>
- Kellogg CA, Griffin DW (2006) Aerobiology and the global transport of desert dust. *Trends Ecol Evol* 21(11):638–644. <https://doi.org/10.1016/j.tree.2006.07.004>
- Kent PT, Kubica GP (1985) Public health microbiology: a guide for the level III laboratory. US Dept. H.H.S. and CDC, Atlanta
- Li S, Kang J, Yu W, Zhou Y, Zhang W, Xin Y, Ma Y (2012) Identification of *M. tuberculosis* Rv3441c and *M. smegmatis* MSMEG\_1556 and essentiality of *M. smegmatis* MSMEG\_1556. *PLoS ONE* 7(8):e42769. <https://doi.org/10.1371/journal.pone.0042769>
- Liu Y, Ning Z, Chen Y, Guo M, Liu Y, Gali NK, Sun L, Duan Y, Cai J, Westerdahl D, Liu X, Xu K, Ho KF, Kan H, Fu Q, Lan K (2020) Aerodynamic analysis of SARS-CoV-2 in two Wuhan hospitals. *Nature*. <https://doi.org/10.1038/s41586-020-2271-3>
- Louveau C, Descroix D, Garnier L, Delamanche I, Chavarot P, Ramière F, Marchal G, Vergnaud G (2005) A nose-only apparatus for airborne delivery of *Mycobacterium tuberculosis* to mice: calibration of biological parameters. *Microbes Infect* 7(3):457–466. <https://doi.org/10.1016/j.micinf.2004.11.019>
- Manlhiot C, Mueller B, O'Shea S, Majeed H, Bernknopf B, Labelle M, Westcott KV, Bai H, Chahal N, Birken CS, Yeung RSM, McCrindle BW (2018) Environmental epidemiology of Kawasaki disease: linking disease etiology, pathogenesis and global distribution. *PLoS ONE* 13(2):e0191087. <https://doi.org/10.1371/journal.pone.0191087>
- McBee ME, Chionh YH, Sharaf ML, Ho P, Cai MW, Dedon PC (2017) Production of superoxide in bacteria is stress- and cell state-dependent: a gating-optimized flow cytometry method that minimizes ROS measurement artifacts with fluorescent dyes. *Front Microbiol* 8:459. <https://doi.org/10.3389/fmicb.2017.00459>
- Mimura K (2002) The trend of *M. Kansasii* infection in Okayama prefecture between 1994 and 2000. *Kekkaku* 77(10):665–669
- Mulay MR, Chauhan A, Patel S, Balakrishnan V, Halder A, Vaish R (2019) Candle soot: journey from a pollutant to a functional material. *Carbon* 144:684–712. <https://doi.org/10.1016/j.carbon.2018.12.083>
- Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA (2007) Manual of clinical microbiology, 9th edn. ASM Press, Washington
- Namkoong H, Kurashima A, Morimoto K, Hoshino Y, Hasegawa N, Ato M, Mitarai S (2016) Epidemiology of pulmonary nontuberculous mycobacterial disease Japan. *Emerg Infect Dis* 22(6):1116–1117. <https://doi.org/10.3201/eid2206.151086>
- Nelson KL, Boehm AB, Davies-Colley RJ, Dodd MC, Kohn T, Linden KG, Liu Y, Maraccini PA, McNeill K, Mitch WA, Nguyen TH, Parker KM, Rodriguez RA, Sassoubre LM, Silverman AI, Wigginton KR, Zepp RG (2018) Sunlight-mediated inactivation of health-relevant microorganisms in water: a review of mechanisms and modeling approaches. *Environ Sci Process Impacts* 20(8):1089–1122. <https://doi.org/10.1039/c8em00047f>
- Noda J, Tomizawa S, Hoshino B, Munkhjarga E, Kawai K, Kai K (2019) Atmospheric dust as a possible survival factor for bioaerosols. E3S Web Conf. <https://doi.org/10.1051/e3sconf/20199904007>
- Ogen Y (2020) Assessing nitrogen dioxide (NO<sub>2</sub>) levels as a contributing factor to coronavirus (COVID-19) fatality. *Sci Total Environ*. <https://doi.org/10.1016/j.scitotenv.2020.138605>
- Pagels J, Wierzbicka A, Fors E, Isaxon C, Dahl A, Gudmundsson A, Swietlicki E, Bohgard M (2009) Chemical composition and mass emission factors of candle smoke particles. *J Aerosol Sci* 40(3):193–208. <https://doi.org/10.1016/j.jaerosci.2008.10.005>
- Poonsuk K, Giménez-Lirola L, Zimmerman JJ (2018) A review of foot-and-mouth disease virus (FMDV) testing in livestock with an emphasis on the use of alternative diagnostic specimens. *Anim Health Res Rev* 19(2):100–112. <https://doi.org/10.1017/S1466252318000063>
- Ramphul K, Mejias SG (2018) Kawasaki disease: a comprehensive review. *Arch Med Sci Atheroscler Dis* 3:e41–e45. <https://doi.org/10.5114/amsad.2018.74522>
- Rasband WS (2018) ImageJ, US National Institutes of Health, Bethesda, Maryland, USA. <https://imagej.nih.gov/ij/>, 1997–2018.
- Richardson H, Rhodes G, Henrys P, Sedda L, Weightman AJ, Pickup RW (2019) Presence of *Mycobacterium avium* Subspecies paratuberculosis monitored over varying temporal and spatial scales in river catchments: persistent routes for human exposure. *Microorganisms*. <https://doi.org/10.3390/microorganisms7050136>
- Rodó X, Ballester J, Curcoll R, Boyard-Micheau J, Borràs S, Morguà JA (2016) Revisiting the role of environmental and climate factors on the epidemiology of Kawasaki disease. *Ann NY Acad Sci* 1382(1):84–98. <https://doi.org/10.1111/nyas.13201>
- Rosen R, Davidov Y, LaRossa RA, Belkin S (2000) Microbial sensors of ultraviolet radiation based on recA<sup>+</sup>::lux fusions. *Appl Biochem Biotechnol* 89(2–3):151–160. <https://doi.org/10.1385/abab:89:2-3:151>
- Ruiz-Gil T, Acuña JJ, Fujiyoshi S, Tanaka D, Noda J, Maruyama F, Querera MA (2020) Airborne bacterial communities of outdoor environments and their associated influencing factors. *Environ Int* 145:106156. <https://doi.org/10.1016/j.envint.2020.106156>
- Russo C, Apicella B, Lighty JS, Ciajolo A, Tregrossi A (2017) Optical properties of organic carbon and soot produced in an inverse diffusion flame. *Carbon* 124:372–379. <https://doi.org/10.1016/j.carbon.2017.08.073>
- Samburova V, Zielinska B, Khlystov A (2017) Do 16 polycyclic aromatic hydrocarbons represent PAH air toxicity? *Toxics* 5(3):17. <https://doi.org/10.3390/toxics5030017>



- Schneider CA, Rasband WS, Eliceiri KW (2012) NIH image to ImageJ: 25 years of image analysis. *Nat Methods* 9:671–675
- Shiraiwa M, Selzle K, Pöschl U (2012) Hazardous components and health effects of atmospheric aerosol particles: reactive oxygen species, soot, polycyclic aromatic compounds and allergenic proteins. *Free Rad Res* 46(8):927–939. <https://doi.org/10.3109/10715762.2012.663084>
- Stanmore BR, Brillhac JF, Gilot P (2001) The oxidation of soot: a review of experiments, mechanisms and models. *Carbon* 39:2247–2268. [https://doi.org/10.1016/S0008-6223\(01\)00109-9](https://doi.org/10.1016/S0008-6223(01)00109-9)
- Tsai DH, Riediker M, Berchet A (2019) Effects of short- and long-term exposures to particulate matter on inflammatory marker levels in the general population. *Environ Sci Pollut Res Int* 26(19):19697–19704. <https://doi.org/10.1007/s11356-019-05194-y>
- van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, Tamin A, Harcourt JL, Thornburg NJ, Gerber SI, Lloyd-Smith JO, de Wit E, Munster VJ (2020) Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. *N Engl J Med* 382(16):1564–1567. <https://doi.org/10.1056/NEJMc2004973>
- Verschoor JA, Baird MS, Grooten J (2012) Towards understanding the functional diversity of cell wall mycolic acids of *Mycobacterium tuberculosis*. *Prog Lipid Res* 51(4):325–339. <https://doi.org/10.1016/j.plipres.2012.05.002>
- Vollmer AC, Belkin S, Smulski DR, Van Dyk TK, LaRossa RA (1997) Detection of DNA damage by use of *Escherichia coli* carrying recA'::lux, uvrA'::lux, or alkA'::lux reporter plasmids. *Appl Environ Microbiol* 63(7):2566–2571
- Wang J, Du G (2020) COVID-19 may transmit through aerosol. *Iri J Med Sci*. <https://doi.org/10.1007/s11845-020-02218-2>
- Wu X, Nethery RC, Sabath BM, Braun D, Dominici F (2020) Exposure to air pollution and COVID-19 mortality in the United States: a nationwide cross-sectional study. Preprint. <https://doi.org/10.1101/2020.04.05.20054502>
- Yoshida S, Saito H, Suzuki H (2011) Present status of studies on epidemiology of *Mycobacterium kansasii*, in special reference to its epidemiology. *Kekkaku* 86(5):515–521

