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Laboratory Study of Effects of Atmospheric Pollutants on Ice Nucleation Activity of Bacteria

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ABSTRACT

Ice nuclei of some bacterial origin as ice catalysts can initiate ice nucleation at temperatures as warm as –2°C in certain laboratory experiments. The ice nucleation activities of airborne bacteria in the real atmosphere may be different from those experiments. To estimate the impact of typical atmospheric pollutants including monocarboxylic acids (MCAs), dicarboxylic acids (DCAs) and ammonia sulfate on ice nucleation activity of *P.syringae pv lachrymans* and *P.syringae pv.panici*, we have conducted some experiments by means of the modified Vali's droplet freezing testing method in the immersion freezing mode with the mixture of the pure water and the polluted water. Our results show that the ice nucleation activity of bacterial origins can be regulated by such pollutant compounds even though the onset freezing temperatures of water droplets mainly depend on the concentrations of ice nucleation-active bacteria. Atmospheric acids can decrease ice nucleation activity of *P.syringae pv lachrymans* and *P.syringae pv.panici*. However, the onset freezing temperatures of water droplets immersed with ice nucleation-active *P.syringae pv lachrymans* will be enhanced by the low concentration of such atmospheric pollutants.

Keywords: Pollutant; Ice nuclei; Droplet freezing test; Immersion freezing; Ice nucleation-active bacteria.

INTRODUCTION

Clouds may consist of ice particles under supercooled conditions. Scientists have recognized their important role in precipitation and the Earth's radiation budget. Ice nuclei (IN) in the atmosphere are required to catalyze cloud ice formation at proper conditions. Since some kinds of bioaerosols can act as ice nuclei even at temperatures warm than –10°C (Schnell and Vali, 1972; Diehl *et al.*, 2001; Iannone *et al.*, 2011; Knopf *et al.*, 2011; Morris *et al.*, 2013; Joly *et al.*, 2014), bioaerosols as an important component of aerosols in the atmosphere have been paid much more attentions over the past decades (Schnell and Vali, 1972; Diehl *et al.*, 2001; Iannone *et al.*, 2011; Knopf *et al.*, 2011; Morris *et al.*, 2013). Ice nucleation-active bioaerosols have widely been found in different regions and climates (Schnell and Vali, 1976; Christner *et al.*, 2008a; Christner *et al.*, 2008b; Pratt *et al.*, 2009; Conen *et al.*, 2011; Garcia *et al.*, 2012; Burrows *et al.*, 2013; Huffman *et al.*, 2013; Monteil *et al.*, 2014; O'Sullivan *et al.*, 2014). Recent numerical studies show that ice nucleation-active bioaerosols can trigger the ice multiplication in the warm-based precipitating shallow cumulus clouds (Ariya *et al.*, 2009; Sun *et al.*, 2010; Sun *et al.*, 2012). Therefore, Ice nucleation-active bioaerosols may play an important role in precipitation and even in climate change.

The ice-nucleation activity (INA) is determined by the size of the nucleating structure of a particular substance (Fletcher, 1963). Proteins in the outer membrane of the bacteria were thought to cause ice nucleation. The highest level of ice nucleation activation is attributed to a protein (lipoglycoprotein) of some bacteria (Green and Warren, 1985), such as *Pseudomonas syringae*. Lipoglycoprotein consists of highly repetitive sequences of amino acids in the bacterial outer membrane that serves as a template for ice crystallization. This IN protein has a closely related infrastructure with a central region, and can be linked to the membrane by a glycosylphosphatidylinositol (GPI) anchor (Govindarajan and Lindow, 1988; Kozloff *et al.*, 1991). The sizes of nucleating insoluble IN protein increase logarithmically with increasing nucleation temperature.

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Govindarajan and Lindow (1988) have confirmed this relationship for bacteria as IN. Frequency refers to the ratio between the numbers of active IN at a given temperature to the number of bacterial cells in a culture. The nucleation threshold temperature and frequency of ice nucleation-active bacteria depend upon the dynamic assembly of multiple, membrane-bound aggregates of ice-nucleating proteins: the larger the aggregate, the higher the supercooling threshold temperature. It has been hypothesized that larger (and more active) sites contain multiple copies of this ice-nucleating protein (Southworth *et al.*, 1988; Lindgren *et al.*, 1989; Ruggles *et al.*, 1993). The relationship between concentration and activity of a bacterial ice nucleation protein is seemingly nonlinear (Southworth *et al.*, 1988).

Effective ice nucleus production ability has been observed in three different bacterial genera, which are mostly Gram negative. They are natural colonizers of plant surfaces. The most important species are *Pseudomonas syringae* and *Erwinia Herbicola* (Hew and Yang, 1992) very common and widely distributed on plant epiphytes and leaves of trees (Maki *et al.*, 1974; Arny *et al.*, 1976; Kozloff *et al.*, 1983; Ercolani, 1991). Much more frequently observed bacteria are *Pseudomonas fluorescens* and *Xanthomonas campestris* (Austin *et al.*, 1978; Obata *et al.*, 1987; Hew and Yang, 1992), normally being residents in soil and water, and even on leaves of trees, pepper and tomato (Austin *et al.*, 1978; Maki and Willoughby, 1978; Warren *et al.*, 1986). Much less frequent are *E. ananas* (Hew and Yang, 1992) and *P. viridiflava* (Obata *et al.*, 1989), which are associated with tea plants and some other crops in Japan (Abe *et al.*, 1989). Other species are *E. uredovora* (Obata *et al.*, 1990), inhabiting erythrina trees (Sutra *et al.*, 1999). Moreover, those kinds of ice-nucleating bacteria occupy high proportions on some kinds of plant leaves, about 41.8% for perennial rye (Austin *et al.*, 1978) and more than 68% for olive (Ercolani, 1991). Another kind of the most active ice-nucleating bacteria, *Pseudomonas borealis DL7*, have recently been found in soil and in the Arctic (Wilson *et al.*, 2006). The predominant ice nucleation-active bioaerosols are *Pseudomonas syringae* and *Erwinia Herbicola* in China. The former distribute mainly in temperate regions such as Beijing, Hubei, Liaoning and Sichuan provinces and the later mainly in subtropical regions such as Guangxi province (Sun *et al.*, 1989). Much more attention *to be paid for Pseudomonas syringae* is attributed to its pathogenicity (Hirano and Upper, 2000), frost damage in plants at higher temperatures (Lindow, 1983), and potential roles in cloud glaciation (Christner *et al.*, 2008b).

Pseudomonus syringae is one of the most effective ice nucleating identified strains to initiate ice formation at temperatures as high as –2°C and is the most efficient nucleating agent yet discovered. It should be emphasized that not every cell is active at a given time and temperature (Hirano and Upper, 2000; Joly, Amato *et al.*, 2014). The fraction of cells as active IN increases with decreasing temperature below –2°C. Activity varies from strain to strain, and also with environmental conditions. Furthermore, for a given strain, the activity frequencies can be influenced by the host plant species. Lindow *et al.* (1982) have shown that there were different activity frequencies between potato and tomato crops at –5°C. Moreover, some studies have shown that the variability of nucleating frequencies is a function of seasonal and diurnal changes for some bacterial populations (Hirano and Upper, 1989). The dead and even the cell-free nuclei of some kinds of bacteria exhibited freezing properties (Anderson and Ashworth, 1986; Phelps *et al.*, 1986). Cochet *et al.* (1994) also have shown that pH negatively influences the bacterial nucleation activity.

However, limited data concerning the impact of atmospheric conditions on the bacterial nucleation activity are available. Recently, the effects of the pH of cloud water, UV light, NO_2 and O_3 on the INA of *Pseudomonus syringae* have been examined (Attard *et al.*, 2012; Sarron *et al.*, 2013). Their results indicated that acidic pH has strong effects on the INA of *Pseudomonus syringae*, which can be highly reduced at the temperatures above –8°C. The anthropogenic activities can even result in the cloud water to be pH 3.0 (database available at http://www.obs.univ-bpclermont.fr/SO/beam/data.php), which is mainly attributed to ammonium sulfate, monocarboxylic acids (MCAs) and dicarboxylic acids (DCAs) (Marinoni *et al.*, 2004). As dominant species of MCAs, formic and acetic acids have been found in the gas phase, aerosols cloud droplets and raindrops (Sun and Ariya, 2006). Most abundant DCAs is oxalic acid, malonic acid and succinic acid in the atmosphere (Sun and Ariya, 2006). Vehicular exhaust and biomass burning are the primary sources of DCAs and MCAs. However, the effects of the mixture of such atmospheric pollutant compounds on the INA of bacteria are still unknown. Therefore, the purpose of this paper is to clarify the lowest concentration of *Pseudomonus syringae* to catalyze the ice formation at temperatures warmer than –20°C and to evaluate the possible impacts of typical atmospheric pollutants on the INA of bacteria.

MATERIALS AND METHODS

Bacteria Strains

Bacterial strains used in this study had been obtained with freezing dried strains included two strains: *Pseudomonas syringae pv.panici* coming from Preservation Centre of Institute of Microbiology, Chinese Academy of Science and *Pseudomonas.syringae pv.lachrymans* coming from Disaster Lab of Chinese Academy of Agricultural Sciences. These two strains were originally isolated from the crop host plants and soils. *Pseudomonas syringae pv.lachrymans* has been recognized as INA bacteria named as PS. *Pseudomonas syringae pv.panici* belong to non-INA bacteria named as PS^0. In our experiments, all freeze-dried bacteria strains were cultured for 1 day at 25°C on plates of nutrient agar and then transferred at least twice for inoculums. Afterwards, the cultured bacteria were incubated at 4°C more than one week prior to processing.

Bacterial suspensions were obtained from 10^8 or 10^9 cells/mL with the aforementioned cultivated bacteria in sterilized ultra-pure Milli-Q water. The low orders of suspension from 10^2 to 10^9 cells/mL were further obtained by means of 10 fold serial dilutions for INA behavior test of suspensions immersed in water droplets.

Mixture of Bacteria and Pollutants

The predominant identified dicarboxylic acids (DCAs) in atmospheric particles are oxalic acid (C2), followed by malonic acid (C3) and succinic acid (C4) (Kawamura and Usukura, 1993; Kerminen *et al.*, 2000). The concentration of DCAs in the precipitation in southern California, US and Tokyo, Japan was 0.9–8.7 µmol/L and 0.1–4.6 µmol/L, respectively (Kawamura and Ikushima, 1993; Sempere and Kawamura, 1994). Based on the concentration of DCAs in the $PM_{2.5}$ in an urban area of Beijing from 2002 to 2003, oxalic acid was dominated in these acids with seasonal average concentrations of 1.2–4.6 µmol/L. Other water soluble carboxylic acids reported include formic, acetic and oxalic acid occupied 2% of the total anion in Beijing urban precipitation (Hu *et al.*, 2005). Thus, we used sodium acetate, oxalic acid and ammonium sulfate as a representative of MCAs, DCAs and inorganic salt, respectively. The decease of the efficiency of bacterial INA in clouds by such compounds may be attributed to the acidification of cloud water, which may denature the tertiary structure of the ice nucleation or impact the bacterial metabolism (Attard *et al.*, 2012). The pH of the solutions has been selected to evaluate their impacts on the efficiency of bacterial INA (Attard *et al.*, 2012). The anthropogenic activities can lead the cloud water to be pH 3.0 (Marinoni *et al.*, 2004), which may require hundred times of the aforementioned normal concentrations of different solutions. Therefore, the concentrations of the solutions for the experiments of bacterial IN are normally set to be much higher than those of the in-situ observations (Attard *et al.*, 2012). In our experiments, the different concentrations of MCAs and DCAs were set to 50, 75, 100 µM and the concentrations of ammonia sulfate were given to 100, 200, 300, 400 µM. On the other hand, Delort *et al.* (2010) presented that the microbial concentration in rain water varied from 10^3 to 10^5 cells/mL. The concentrations of bacterial suspensions in our experiments were chosen from 10^4 to 10^6 cells/mL.

The Scenario of a Single Pollutant Mixed with Bacteria Suspensions

To evaluate the effect of different pollutant conditions on the INA of PS and PS^0, the mixture solution was conducted with the mixture of 9 mL specific concentration of single pollutant solute with 1 mL different orders of magnitude for bacterial suspensions to ensure the bacterial concentrations in the mixed solutions being either 10^4 or 10^6 cells/mL (Table 1).

The Scenario of Compound Solutions Immersed with Bacterial Suspensions

To evaluate the impact of compound solutions on the INA of PS and PS^0, the INA experiments for bacterial suspensions mixed with compound solutions had been performed. Three types of compound solutions (M1, M2, M3) mixed with different orders bacteria suspension were used. As a result, this scenario (Table 2) results in the bacterial concentration of droplets to change between 10^6 to10^3 cells/mL and keep the mixed solutes concentration of M1, M2, M3.

Apparatus for the Measurement of Ice Nucleation Activities

The INA of droplets for the scenario a and b were measured by a homemade droplet freezing test apparatus developed by Yang and Feng (2007) based on the method of Vali (1971). The apparatus can automatically detect droplet freezing signals and process test data, which consists of a cold plate cooled by a set of thermocouples. The cold plate has 47 thermosensitive cells equally distributed on its surface to position water droplets and monitor the temperature of

Table 1. The mixed contents for the scenario a:

	PS		PS^0	
	10^4 cells/mL	10^6 cells/mL	10^4 cells/mL	10^6 cells/mL
sodium acetate(SA) 50 µM	+	+	+	+
oxalic acid(OA) 50 µM	+	+	+	+
ammonium sulfate (AS) 100 µM	+	+	+	+

Note: + represents the ice nucleation experiment having been conducted with one kind of bacteria mixed with one kind of solutions. PS and PS^0 denote *Pseudomonas syringae pv.lachrymans* and *Pseudomonas syringae pv.panici,* respectively.

Table 2. The droplets for the scenario b:

Cells/mL	M1			M2			M3		
	SA	OA	AS	SA	OA	AS	SA	OA	AS
	100 µM	100 µM	200 µM	50 µM	50 µM	100 µM	25 µM	25 µM	50 µM
PS/PS^0 10^3		+			+			+	
PS/PS^0 10^4		+			+			+	
PS/PS^0 10^5		+			+			+	
$PS/PS^0 10^6$		+			+			+	

Note: + represents the ice nucleation experiment having been conducted with one kind of bacteria mixed with one kind of mixed solutions. SA, OA and AS denote sodium acetate, oxalic acid and ammonium sulfate, respectively, PS and PS^0 denote *Pseudomonas syringae pv.lachrymans* and *Pseudomonas syringae pv.panici*, respectively.

them. During the freezing experiments, the droplets were linearly cooled at a certain cooling rate controlled by a 818P4 Eurotherm temperature controller. The freezing latent heat released by each freezing droplet will be detected by the thermosensitive cell, and then transformed into a voltage signal by the arithmetic circuitries. A series of identical signals produced by all freezing droplets will be real-time monitored by a computer. The differential and accumulative concentration temperature spectra of ice nuclei will be obtained and analyzed by a software. For each suspension solution, 235 droplets (i.e., five times of 47 droplets for repetitions) were measured to satisfy the statistical demand. We know that the droplet size can also impact the ice nucleation temperatures of INA. The shape of droplets titrated by a pipette with the sizes no larger than 10 µL on the plate can stay spherical for relatively a long time so that the manipulation of immersion of the INA can be done easily. The droplet size of 10 µL was conducted in our experiments as the same size as Vali and Stansbury (1966). The relative humidity in the lab was around 70%. The initial temperature was set to 0°C and then decreased at the rate of 2°C per minute until –30°C.

Algorithm to Determine the Cumulative Nucleus Concentration

The lowest effective accumulated ice nuclei concentration is needed to represent the INA of bacterial suspensions at a specific temperature of *T* °C. The cumulative ice nuclei concentration per bacterial suspension at the temperature of *T* °C was calculated according to the approach given by Vali (1971) as the following equation:

$$K(T) = [\ln(N_0) - \ln(N_T)]/V \quad (1)$$

$$B(T) = [\ln(N_0) - \ln(N_T)]/A \quad (2)$$

where, K(*T*) and B(*T*) represent the accumulated nuclei concentration of the per measured solution droplet and the per bacterial cell, respectively. N_T is the number of the unfreezing droplets at T°C. N_0 is the initial total number of droplets. *V* is the droplet volume. *A* is the number of bacterial cells among each of the test droplets.

Statistical Analysis

We calculated the mean and standard deviations of the freezing temperatures for the tested samples. For each test sample, the droplets are more than 235. The test error lines were drawn depending on the standard deviations. All the experimental results were statistically analyzed with the statistical analysis software SPSS version 10.0 for the T test, and the significance level α was set to 0.05.

RESULTS AND DISCUSSION

Ice Nucleation Activity of Bacteria

Under our experimental conditions, PS^0 did not display any expected INA: the freezing temperatures were –19.9 ± 3.3°C. The concentration of the bacteria of their tested suspension was 10^8 cells/mL (OD_{600} = 0.25), which did not show any significant difference from the freezing temperatures of the pure water droplets (–20.8 ± 2.7°C). However, PS (*Pseudomonas syringae pv.lachrymans*) presented higher freezing temperatures around –5.0 ± 0.8°C with the same concentrations of bacterial suspensions. Furthermore, the freezing temperatures for PS suspensions appeared to be concentration-dependent. With the PS suspension concentration decreasing from 10^9 to 10^2 cells/mL, the droplet freezing temperatures changed from –2.8 to –25.8°C. Under the low concentration of 10^4, 10^3 and 10^2 cells/mL, the tested droplets' freezing temperatures were changed from –7.2 to –24.6°C, –8.0 to –25.6°C, –9.4 to –25.8°C, respectively (Fig. 1), which indicates that the low concentration of INA bacteria cannot catalyze the ice formation at the relatively warm temperatures. Furthermore, the nucleated droplets decrease sharply at temperatures above –20°C if the PS concentrations are less than 10^4 cells/mL. The nucleation tendency of the tested droplets containing PS even becomes similar to that of water droplets without bacteria when the PS concentrations are less than 10^3 cells/mL. The droplets nucleated should contain at least above 100 PS in each droplet (Nejad *et al.*, 2006). The PS concentration of the tested droplets with 10^4 CFU/mL is less than 100 cells in each droplet. For the samples with PS concentrations less than 10^3 CFU/mL, the PS cells tend to be less than 10 for each droplet so that such droplets show less active. These results are not consistent with those reported by Mortazavi *et al.* (2008). They showed that the low concentrations between 10^4 cells/mL and 10^2 cells/mL can also initiate droplet glaciations processes at temperature above –10°C (see Fig. 5, Mortazavi *et al.* (2008)). We still cannot clearly figure out reasons for those different results. It may be possible due to the different type of PS to be tested. However, further tests are necessary to clarify the causes.

As for the IN activity for a single cell under the different PS bacterial suspension concentrations of 10^8, 10^7, 10^6, 10^5, 10^4 cells/mL, the corresponding cumulative ice nuclei concentration per bacterial cell at the same freezing temperature –7°C were $10^{-5.36}$, $10^{-4.70}$, $10^{-4.09}$, $10^{-3.99}$ and $10^{-3.86}$. The higher concentration of bacterial suspensions results in the lower IN amount needed for each cell to be activated. With the further lower concentration of bacterial suspensions , such as 10^3 and 10^2 cells/mL, the single bacterial cell can produce $10^{-2.92}$ and $10^{-2.37}$ IN, respectively, if droplets immersed with those amounts of cells were frozen at the freezing temperature –9.4°C. However, the no existence of frozen droplets above this temperature in our experiments indicates that the orders of magnitude of those bacterial concentrations cannot produce such high IN concentrations at temperatures warmer than –9.4°C.

Effects of Atmospheric Pollutants on Bacterial Ice Nucleation Activity

The Effects of Single Organic and Inorganic Pollutant on Bacterial Ice Nucleation Activity

Simulating the polluted air conditions with the artificial water solutions as described in Table 1, we investigated the effects of typical atmospheric pollutants on the INA of

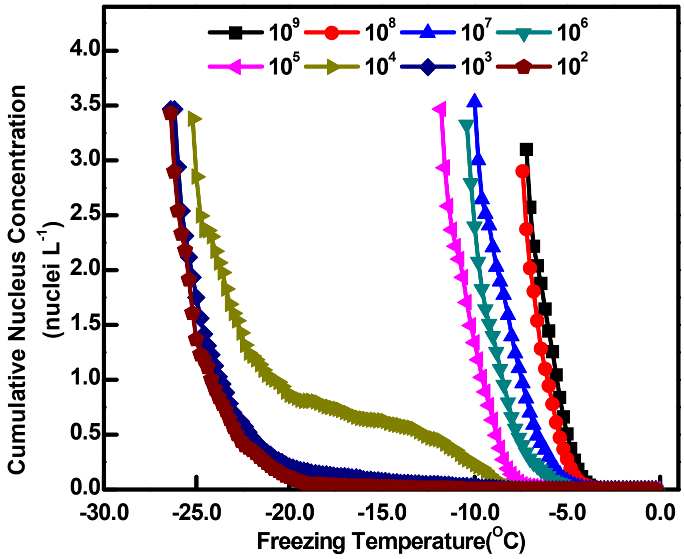

Fig. 1. Ice nucleation spectra of tested bacteria *Pseudomonas syringae pv.lachrymans* (lgc denotes the logarithm of the bacteria concentration).

PS. Fig. 2 indicates that atmospheric acids can decrease the INA of PS mixed only with a single typical atmospheric pollutant (Fig. 2) no matter under a high concentration of PS (10^6 cells/mL) or a low concentration of PS (10^4 cells/mL). Furthermore, those atmospheric acids can also decrease the freezing temperatures of droplets immersed with PS^0 strain. These results are consistent with the findings by others (Cochet *et al.*, 1994; Attard *et al.*, 2012). Cochet *et al.* (1994) found that the pH control of culture negatively impacted the INA of PS. Recently, Attard *et al.* (2012) showed that the lower pH values of tested water droplets have, the stronger reduced INA of PS will be for most of tested PS.

The Effects of the Mixed Pollutants on the INA of PS and PS^0

To investigate effects of the mixed pollutants on the INA of bacterial suspensions. Serial experiments of bacterial cells with the mixture of complex organic and inorganic substances (Table 2) had been performed. Fig. 3 showed the average freezing temperatures for PS and PS^0 under the different orders of magnitude with the mixture solutions of compound pollutants. In these experiments, we further assumed that there are no chemical reactions among the three mixed solutions. The results show that the high concentrations of the mixed pollutants, such as the cases of M1 and M2 can decrease the INA of PS when the concentrations of PS are greater than 10^4 cells/mL, whereas such high concentrations of the mixed pollutants cannot obviously regulate the freezing temperatures of droplets immersed with PS^0 since the mixed pollutants for the case M1 cannot decrease the freezing temperatures of droplets immersed with PS^0. It is worth to note that the low concentrations of the mixed pollutants of the case M3 can improve the INA of PS no matter how many concentrations of PS suspensions in the solutions. Furthermore, this tendency will be more prominent under the low concentrations of PS suspensions. However, this phenomenon did not occur in the experiments for PS^0 suspensions except for the case with the highest PS^0 population (10^6 cells/mL).

Ice initiation at temperatures warmer than –10°C have been paid much attention in the cloud physics (Sun *et al.*, 2012). The pollutant concentrations and the PS concentrations both determine droplet glaciations at temperatures above or below –10°C. With a relatively high PS concentration at 10^6 cells/mL, the freezing temperatures of the droplets are all above –10°C no matter how much the concentration of the mixed solution will be. The high concentration of the mixed solution can result in the freezing temperature to be less than –10°C as the PS concentration is in the orders of magnitude 10^5 cells/mL. The low concentration of the mixed solution can lead to the freezing temperature to be close to –10°C as the PS concentration is in the orders of magnitude 10^4 cells/mL. Since the concentrations of the INA of PS in the real atmosphere are relatively low and the mixture of organic and inorganic pollutants in the real atmosphere with low concentrations may enhance the ice formation, such an effect should be considered in the estimation of the role of bioaerosols for the cloud ice formation in the atmosphere.

The microorganisms are typically attached to the other particles in the atmosphere. Hence, the INA of bioaerosols will be influenced by the compositions of particulate organic matters. It has been reported that the organic aerosols occupied a significant fraction (20–90%) of the atmospheric mass concentration of total aerosols (Kanakidou *et al.*, 2005). Among the organic aerosols, the low molecular weight carboxylic acids involve several MCAs and DCAs. As for MCAs, formic, acetic, lactic and propanoic acids are the dominant species. Similarly, oxalic, malonic (C3), succinic (C4), Maleic and Glutaric (C5) acid are considered the main components of DCAs. Furthermore, MCAs are much more abundant than DCAs in the atmosphere and MCAs typically accounted for about 71% of total organic carboxylic acid and DCAs only accounted for 29% of them. Recent

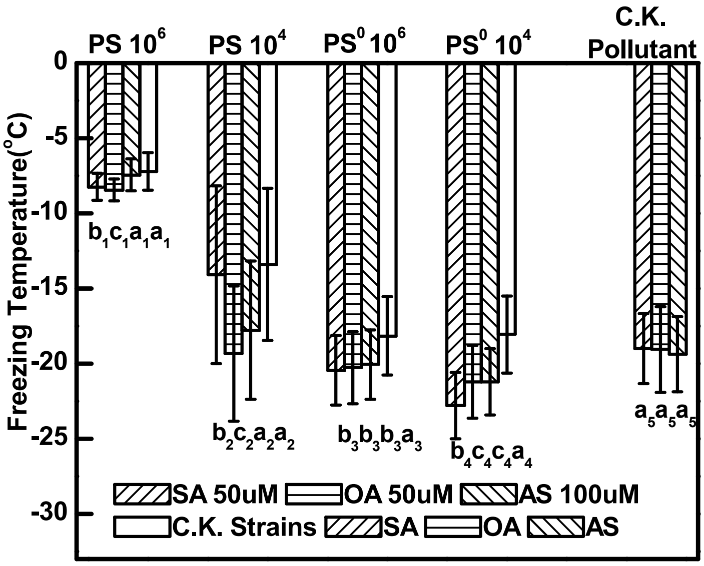

Fig. 2. Freezing temperatures of mixture of typical air pollutants and bacteria strain (the same letters in the panel mean no statistically significant difference between two ice nucleation tests at the 0.05 level for Dunnett's Test, vise versa).

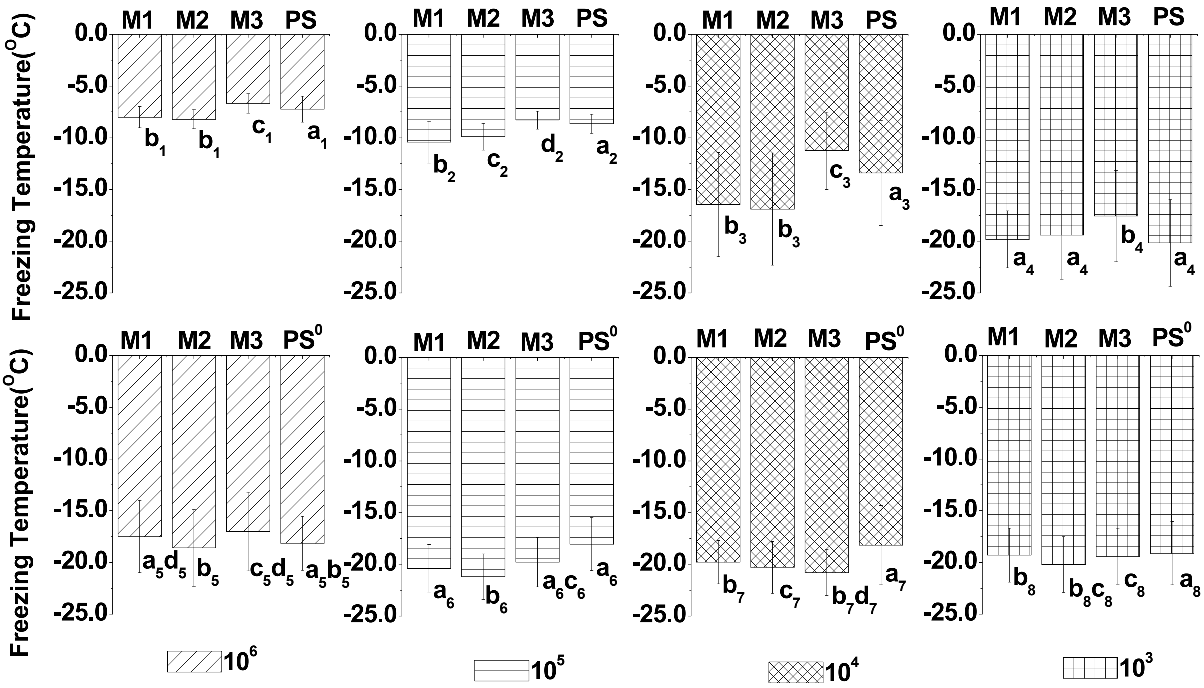

Fig. 3. Freezing temperatures of INA bacteria PS & non-INA bacteria PS^0 mixed with different concentrations of typical mixture air pollutants (the same letters in the panel mean no statistically significant difference between two ice nucleation tests at the 0.05 level for Dunnett's Test, vise versa).

field studies on atmospheric aerosols have shown that the low molecular weight carboxylic acids always constitute an important fraction of fine aerosols and organic aerosols are an important part of the global cloud condensation nuclei budgets (Acker *et al.*, 2002).

Soluble DCAs and MCAs may be good candidates for cloud condensation nuclei in the atmosphere (Sun and Ariya, 2006). Insoluble organic compounds may act as ice nuclei through heterogeneous nucleation. Some laboratory results have indicated that the low molecular weight soluble DCAs therefore do not play an important role in ice freezing processes (Du and Ariya, 2008; Mortazavi *et al.*, 2008). Zobrist *et al.* (2006) measured five low molecular weight carboxylic acids (oxalic, adipic, succinic, phthalic, fumaric) with the differential scanning calorimeter and concluded that only the dehydrate of oxalic acid can serve as a heterogeneous ice nuclei. It is well known that each kind of pollutants rarely exits purely in the atmosphere. Therefore, the study of the effects of the internal mixture of the soluble pollutant compounds, such as sodium acetate, oxalic acid and ammonium sulfate on the INA of bioaerosols is helpful for us to understand the role of bioaerosols in the cloud ice formation.

CONCLUSIONS

As the representative of common bacteria strain, PS^0 cannot act as effective ice nuclei no matter how much concentration is under different pollutant scenarios. On the contrary, as an INA strain, PS showed high ice nucleation capability if there are enough concentrations. It should be noted that the low concentration of the mixed organic solution can enhance the INA of PS. Such enhancements occurred in all our test cases with different concentrations of PS from 10^3 cells/mL to 10^6 cells/mL, especially prominent for the low concentration of PS. Since the INA of PS depends on the ice nucleation protein located in their outer membrane, the mixed solutions may change the ice nucleation protein of PS. However, the mechanisms for such an adjustment are not still well known, especially for the enhancement of INA of PS with a relatively low concentration of the mixed solutions.

In fact, even now how the relevant microscopic properties of the ice nucleation proteins of PS determine its macroscopic ice nucleating efficiency is still little known. Weidner (2013) presented that the water molecules at the ice-nucleating protein surface quickly became more ordered and the molecular motions became inactive. They also found that thermal energy could be removed from the surrounding water efficiently. Weidner (2013) then suggested that ice nucleating proteins might have a specific mechanism for heat removal and ordering water molecules at low temperatures. The study for this mechanism is still underway. The phenomenon that the low concentration of the mixed organic solution can enhance the INA of PS cannot be explained by our current knowledge. The priority for this understanding is to make the above mechanism clear, and then we may figure out how the low concentration of the mixed organic solution affects this mechanism and subsequent freezing processes. Moreover, atmospheric ice nucleation is a very difficult process to be performed in the laboratory with the precise manipulation under experimental conditions. Therefore, further studies are needed with collaborations between different disciplines.

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