THE DEVELOPMENT AND UTILIZATION OF A NEW DROPLET FREEZING ASSAY

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THE DEVELOPMENT AND UTILIZATION OF A NEW DROPLET FREEZING ASSAY

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For my unconditionally loving and supportive family.

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LIST OF SYMBOLS AND ABBREVIATIONS

AIDA	Aerosol Interaction and Dynamics in the Atmosphere
ATD	Arizona Test Dust
CEC	Cloud expansion chamber
CCN	Cloud condensation nuclei
CCNc	Cloud condensation nuclei counter
CFDC	Continuous flow diffusion chamber
CNT	Classical Nucleation Theory
DFA	Droplet freezing assay
DSC	Differential scanning calorimeter
GT-DFA	Georgia Tech Droplet Freezing Assay
HULIS	Humic-like substances
IN	Ice nuclei
J_{het}	Heterogeneous freezing rate
J_{hom}	Homogenous freezing rate
MCSM	Multi-Component Stochastic Model
MICC	Manchester Ice Crystal Chamber
N	Total number of particles suspended in a droplet
N_{ice}	Number of frozen droplets
n_s	Number of active sites at a given temperature
N_{TOT}	Total number of samples/droplets
RH_i	Relative humidity with respect to ice
s	Unit surface area
SCSM	Single Component Stochastic Model

SS	Supersaturation
SS_{50}	Supersaturation at which 50% of the sampled particles have nucleated
Δt	Time
T	Temperature

SUMMARY

A droplet freezing assay (DFA) was developed for the testing of ice nucleation of various atmospheric particles. The primary focus within this thesis is on the development, calibration, and testing of bacterial samples; there is a particular interest given to finding a standard operating procedure for testing bacteria with DFAs, especially between the atmospheric and biological communities. Results showed that current methods create a clear bias in experimental data, and has implications for the treatment of bacteria as IN in global and regional climate models.

CHAPTER 1

INTRODUCTION

Ice Nucleation: Processes, Theories, and Common Ice Nuclei

Clouds that contain water in the ice phase can have large impacts on the processes that occur within said cloud, whether it is fully glaciated or is mixed in phase. Ice that is present in clouds can quickly initiate precipitation processes[4], and thermodynamically ice formation in clouds can invigorate convective storms[5]. Fully glaciated clouds at higher altitudes, such as cirrus, cirrostratus, etc., have a net warming effect on the atmosphere, negating part of the radiative cooling effect caused by their lower level, liquid counterparts[5, 7]. A simple, direct, and accurate relationship between ice phase clouds and climate does not currently exist for models; while laboratory studies have been carried out on various ice nuclei (IN), in a range of nucleation modes[8, 9], it is important that future work stays focused on experiments that are most relevant to the atmosphere so that understanding of the role in the climate of icy clouds in the climate system forward holistically. Part of this process involves streamlining and standardizing of methods for testing IN activity between all communities involved, so that results can be compared more easily and variances between experimental results can be more easily identified and dealt with. Another piece though, will be continually assessing old and new data to determine what the most accurate representations of the atmosphere are in laboratory experiments. This thesis is an attempt at contributing in some small part to that those goals.

Ice Nucleation Pathways

Ice nucleation can occur through one of five main pathways: homogenous, deposition, immersion, condensation, and contact freezing. Homogenous freezing occurs in pure droplets (i.e. no IN are present in the water), and occurs around -36°C to -40°C. During deposition freezing, water deposits directly onto an IN from the vapor phase. Through immersion mode, a particle activates as a cloud condensation nuclei (CCN) at a temperature above freezing, and later activates after sufficient cooling has occurred. Similarly, condensation freezing occurs within a liquid droplet, however the droplet forms and freezes after the IN has reached conditions below freezing. Finally, in contact

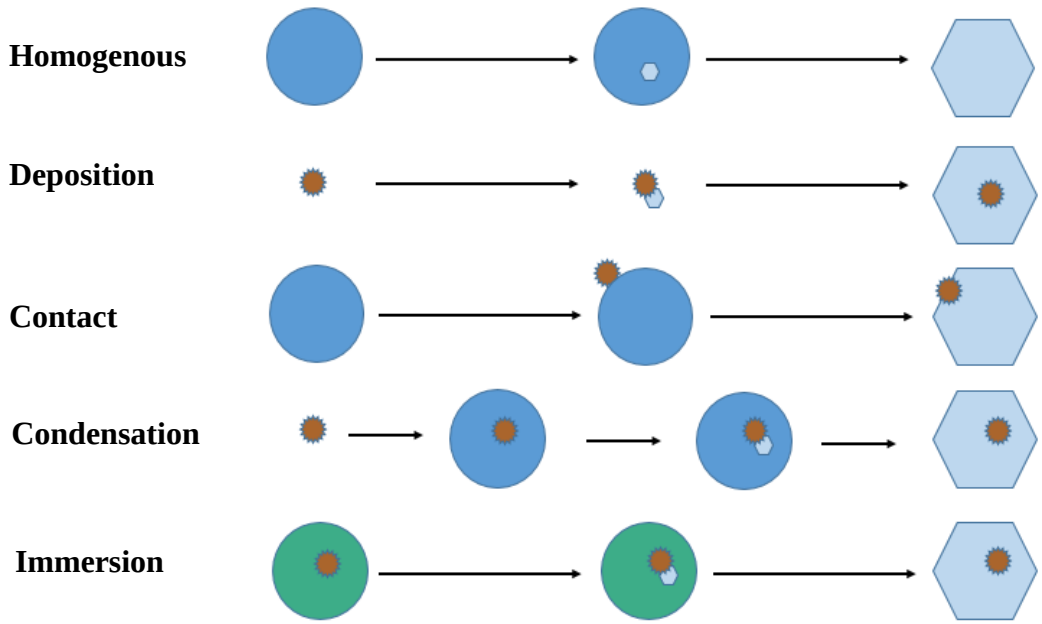

Fig. 1.1 — Visual depiction of freezing pathways, including both homogenous and heterogeneous modes. Names for each are included to the left of the pathway.

mode, a particle or some other object collides with a supercooled water droplet, causing the droplet to freeze[4, 8]. A visual depiction of these pathways is illustrated in Figure 1.1. Each pathway has its own optimal conditions at which it is most likely to occur; for example, condensation mode is more likely to occur at a higher relative humidity with respect to ice (RH_i) and at temperatures around -20°C, while deposition mode requires a

much lower temperature (typically below -30°C), and still requires a high RH_i[8]. The majority of this work will focus on immersion freezing; due in part to the community's consensus on the importance of immersion freezing to mixed-phase clouds[8, 9], as well as the limitations of the instrument created for our IN experiments[9] which will be discussed further in Section 1.3.5

Nucleation Theory

Different theories can be applied to why and when a particular droplet will freeze, depending on the conditions surrounding a given nucleation event. The method and theory chosen to analyze data affects both assumptions made about the IN properties of the material being tested, as well as any model predictions of IN. Depending on which theory is given more weight in an experiment, the nucleation events can be interpreted in different ways and can affect assumptions on the relative importance of various IN, making proper selection of an ice nucleation theory for data analysis and model usage imperative [4, 11-13]. In early nucleation experiments, what is now referred to as classical nucleation theory was the primary method used[4, 8, 9]. However, as more work was conducted on a wider range of experimental conditions, other theories—the singular and multi-component stochastic theories—began to evolve as well.

Classical Nucleation Theory

Classical Nucleation Theory (CNT) assumes that ice nucleation is a wholly random and stochastic process, meaning freezing events are solely dependent only on the amount of time a droplet spends at a given temperature. CNT is typically applied to pure water droplets, but with the addition of ice nuclei to the droplet it becomes the Single Component Stochastic Model (SCSM) which states IN activity of a droplet with nuclei present will increase over that of a pure water droplet, although freezing is still primarily dependent on the amount of time spent at a given temperature[11-13]. Equations (1) and (2)

show the calculations for droplets frozen at a specific temperature, where J_{het} and J_{hom} are the heterogeneous and homogenous nucleation rates respectively, s is the unit surface area of the nucleating particle(s), and Δt is time spent at a given temperature.

$$f_{ice} = 1 - \exp(-J_{hom} \cdot V \cdot \Delta t) \quad (1)$$

$$f_{ice} = 1 - \exp(-J_{het} \cdot s \cdot \Delta t) \quad (2)$$

Equation (1) states that the higher the nucleation rate, the larger the volume, and the longer a droplet spends at a given temperature, the more nucleation events will occur. Similarly, equation (2) shows the number of droplets that freeze will increase if the nucleation rate is higher, the surface area of the particles is larger, or the amount of time spent at a temperature is longer. Though, given that the total surface area present within a droplet can increase as droplet volume does, the effect of droplet size cannot be ignored within equation (2)[4, 13, 14].

If it cannot be assumed that all individual particles of the nucleating material have the same size and morphology, the surface area variable can be further simplified by letting the total surface area, *s*, be equal to the surface area of an individual particle and then multiplying this by the number of particles, N, in the droplet. This modified equation becomes part of the Multi-Component Stochastic Model (MCSM) given its assumption of non-uniformity of the particles within a droplet and is discussed further in section 1.3.3. The derivation for both equations (1) and (2), and the modified form of (2) are in Appendix A.

The Singular Model

The Singular Model assumes that each IN has a specific number of active sites that cause nucleation to occur at a temperature specific to that site (i.e. if each particle in a sample of IN has the same active site, the droplets they are in will all freeze simultaneously). Unlike CNT and SCSM, this model ignores any potential time

dependence in the nucleation process[11]. Equation (3) shows the calculation for the frozen fraction of droplets using the Singular Model, where n_s is the number of active sites at a given characteristic temperature, T.

$$f_{ice} = \frac{n_{ice}}{n} = 1 - exp(-n_s(T)s) \quad (3)$$

The full derivation for this equation can be found in Appendix A.

The Multi-Component Stochastic Model

The Multi-Component Stochastic Model combines components of both the Singular Model and SCSM, allowing for the effect of time dependence, as well as accounting for the presence of specific active sites [11]. Several different models have been created to describe this mode of thinking, one of the most prominent of which is the Soccer Ball Model[12]. It assumes each potential ice nuclei is similar soccer ball in that it has many "faces" that can act as ice nucleation sites. Each of these sites can have different IN efficiencies (applying MCSM), or the same efficiency. Assuming the latter essentially creates one nucleation site on each particle (taking the model back to the Singular Model).

Common Ice Nuclei

Dust

Dust is one of the largest global sources of IN, being emitted to the atmosphere at rates of up to 3000 Tg per year[9]. Additionally it has been shown that the largest source of IN for immersion freezing in the majority of the free-troposphere is mineral dust[15]. Additionally, several common mineral components of different types of dust have been shown to be efficient IN in temperature ranges from approximately -36°C to -15°C,

which covers the range of temperatures most relevant to the immersion and condensation freezing modes[8].

While many laboratory studies have focused on desert dusts, natural dust proxies (such as Arizona Test Dust, ATD), or single mineral components of dust up to this point, alternative and more complex types of dust as potential sources of IN, such as agricultural soil[8, 9], or chemically processed dust[16] have begun to garner more interest recently.

Bacteria and Other Biological Material

Bacteria

Bacteria have been shown to be extremely efficient IN, especially in immersion freezing mode[8, 9, 17]. However, experimental methods vary widely between studies, especially when comparing methods in the biological versus atmospheric communities. In papers from the atmospheric community, such as Broadley, et al., 2012, Welti, et al., 2012, Budke and Koop, 2014 (to name a few), atmospheric conditions have been well accounted for within experiments—including cooling rates, droplet sizes, etc. However, the main biological IN tested in these experiments is Snomax® (referred to as Snomax from this point on), a strain of Pseudomonas syringae that is optimized to express the inaZ gene, and has furthermore been lyophilized, creating a sample that is optimized to produce ice, and is extremely different from how the same bacteria would be found in the natural environment[20].

Studies that have come from the biological community do test and classify various isolates of bacteria found in the atmosphere; however, the droplet sizes used in these studies are far too large to be representative of actual droplets in the atmosphere,

with volumes as large as 120μL[21-25]. Besides the fact that these volumes are completely unrepresentative of droplet sizes in actual clouds (average volume is in picoliters), the large volume used for these experiments can bias the freezing temperatures measured for the samples. Given equation (1), or equation (6) in Appendix A, and as discussed in section 1.2.1, the larger the volume of a droplet, the more material can be present within it, thereby increasing the total surface area of the material in the droplet and statistically increasing the chances of an extremely efficient IN site being present[4, 13, 14]. Additionally, these larger volumes are often tested inside of centrifuge tubes in place of on well plates with a flat surface, causing unnecessary connection between the liquid and the surfaces of the container. This thereby increases the chances of a nucleation event occurring due to initiation of ice formation on any surface imperfections (divots, scratches, pores, etc.) present on, as well as sample contact with, the sides of the container, in the same way that these types of surface imperfections induce nucleation on dust particles, etc.[9, 26-29]

Bacterial Fragments and Other Bioaerosols

Other relatively common ice nuclei include fertile soil, fungi, pollen, and soot particles[8, 17, 30, 31]. Fertile soil—which for experimental purposes is dust mixed with organic and biological material—has been shown to have higher nucleation temperatures than pure dust, with nucleation behavior usually dominated by fertile soil above -15°C [30, 32, 33]. These studies have shown the biological material (e.g. HULIS, surface proteins, bacteria and bacterial fragments, etc.) is the factor contributing to increased activity using heat and other treatments to destroy IN active biological material in the samples. However, which of the materials specifically contributes the most to IN activity has yet to

be concluded with the current studies[30, 32, 33]. Regardless, this has especially important implications for areas with high land usage and varying vegetation, as a changing climate and the over extension of resources could cause these locations to be large sources of highly IN active dust in the future[34, 35]. Fungi are some of the most prevalent bioaerosols in the atmosphere, although less work has been dedicated to the IN activity of fungi than other bioaersols thus far. Results published in the literature report a freezing range between -4°C and -30°C[8]. Similarly, pollen has shown a range between -5°C to -30°C[8, 36-38].

Measurement Techniques

There are several techniques to measure the ice nucleation activity of aerosols, all of which have their own advantages and disadvantages. Instruments like cloud chambers are labor intensive and expensive to create, but allow for the measurement of the full spectrum of nucleation types, over a much wider range of nucleation conditions (i.e. RH_i, temperature, time elapsed at given conditions, etc.) than allowed by other methods[9]. Droplet Freezing Assays (DFAs) on the other hand, are relatively cheap and easy to build, although typically only allow for the measurement of immersion freezing of droplets, and are limited in their scope of producing realistic cloud conditions [9]. Other, more sophisticated instruments have the potential to create very controlled experiments that also involve the least studied nucleation mode, contact freezing; they are expensive, and the experiments can be difficult to execute, but their results offer unique insight into a less understood freezing process[9]. Depending on the goals of the researcher, each method and instrument has its own benefits for the given project.

Cloud Chambers

Cloud expansion chambers (CECs) generate cloud-like conditions and nucleate particles through adiabatic expansion[9]. CECs can test immersion, condensation, and depositional freezing, and provide a more realistic environment within the experiment. However, these instruments are rather large, expensive, and difficult to build given the many variables they have to control, and the many measurements they take. For example, the Aerosol Interaction and Dynamics in the Atmosphere (AIDA) chamber at the Karlsruhe Institute of Technology has various instruments that measure different variables like particle, droplet, or ice crystal number, chemical composition of aerosols, as well as hygrometers, a dew point mirror, and other typical instrumentation to measure the conditions within the chamber at any given time[8, 39]. The Manchester Ice Crystal Chamber (MICC) at The University of Manchester has many of the same instruments, although its dimensions are slightly different[40]. While these chambers can provide robust analysis for IN experiments, it is less accessible for those looking to build their own instrument.

Continuous Flow Diffusion Chambers

Continuous flow diffusion chambers (CFDCs) measure ice nucleation by utilizing a flow through a chamber that contains temperature controlled parallel or concentric walls that are coated in thin layer of ice. The relative humidity of the chamber is controlled by altering the temperatures of the plates[9]. CFDCs allow for a large total volume of sampling for immersion, condensation, and depositional freezing in a relatively short period. However, the brief sampling time through the instrument is also

what makes the capture of any time dependencies in the freezing events within the experiment difficult. Results with CFDCs are therefore more relevant to singular freezing, rather than for both singular and stochastic freezing.

Instruments for Measuring Contact Freezing

Contact freezing is one of the most difficult modes to test, due to the level of control needed over the environment and droplets. Instruments like acoustic levitators and optical tweezers have been useful in this regard, as they allow for climate control of the droplets or individual droplet, while also removing many variable elements from the experiment[38, 41]. However, given that these instruments are rather expensive, few studies thus far have focused on contact freezing, creating not only a lack of data, but also limited knowledge on any biases that may be inherent to the use of these tools.

Droplet Freezing Assays

Droplet Freezing Assays (DFAs) have been utilized in ice nucleation experiments since the IN activity of melted snow and hail samples were tested in a DFA composed of a copper cold stage overlaid by an oil coated aluminum sheet at University of Wyoming[42]. Other DFAs were developed over time, including iterations of the Vali et al cold stage mentioned, as well as entirely separate designs. Several groups created DFAs that housed microcentrifuge tubes inside of a cooling bath[9, 43-45]; others created instruments that were a playoff of the original design by Vali et al, called a Differential Scanning Calorimeter (DSC), where frost growth and evaporation is prevented by coating droplets with oil after being placed onto a cold stage[3, 46, 47].

One of the more common instruments built and used for ice nucleation experiments, all iterations of the DFA are relatively inexpensive and easy to build; however, all versions also have the major drawbacks of 1.) only being capable of immersion freezing measurements, and 2.) creating a potential bias through contact with the plate the droplets are placed on[9]. While it is impossible to work around the freezing mode limitation, other negative impacts can be mitigated through specific design elements. For example, DFAs such as uL-NIPI and BINARY create minimal contact between the droplets and glass plates they sit on (using a contact angle of at least 100° with 1μL droplets)[14, 19, 48], rather than testing samples in centrifuge tubes which contact the sample on all sides and largely decrease the representation of an actual droplet in shape and size in their experiments. Evaporation of the droplets and the Wegener–Bergeron–Findeisen process can be circumvented by arranging samples in individual cells and flushing the chambers housing the instruments with dry nitrogen gas, rather than creating an oil-water interface with the droplets, as with DSCs. The Georgia Tech DFA (GT-DFA) was designed with these concepts in mind, and is described in detail in Chapter 2.

CHAPTER 2

GEORGIA TECH DFA

Drawing from the designs of other groups and their instruments, and utilizing what was available already, a DFA was designed for operation at Georgia Tech. Like the aforementioned DFA's, the GT-DFA measures immersion freezing of droplets using a multi-well plate on top of a temperature controlled cold plate, in conjunction with a camera set-up to record experiments. A more detailed description of the instrument and initial control experiments are described within the following sections.

Instrument Design

The GT-DFA, depicted in Figure 2.1, was built based on the design of other DFAs such as BINARY and uL-NIPI[14, 19], and was modified from a protein crystallization instrument[49]. It consists of an aluminum plate connected to a refrigerated chiller (ANOVA; A40) which controls both the temperature and cooling rate of the instrument. The plate housed in a sealable Teflon chamber, which is purged with clean, dried lab air (RH ~ 5%) for an hour before the plate is cooled to prevent condensation and frost formation within the chamber during experiments. This step is critical to prevent the occurrence of the Wegener–Bergeron–Findeisen process, where water or frost in the surrounding environment causes early nucleation of the droplets due to deposition of water vapor[4, 50]. Additionally, this process can be initiated by an already frozen droplet causing water uptake onto the frozen droplet at the expense of the surrounding

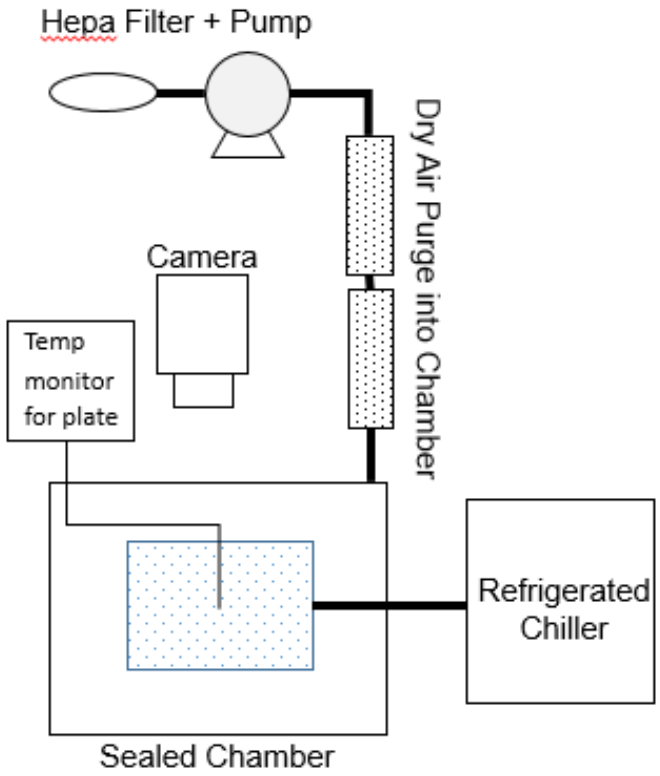

Fig. 2.1—Schematic of the GT-DFA set up. Coolant circulates between the aluminum plate and the refrigerated chiller at a prescribed rate, while the sealed chamber is continuously purged with dry air (RH ~0.05%) and a camera continuously monitors the status of the droplets.

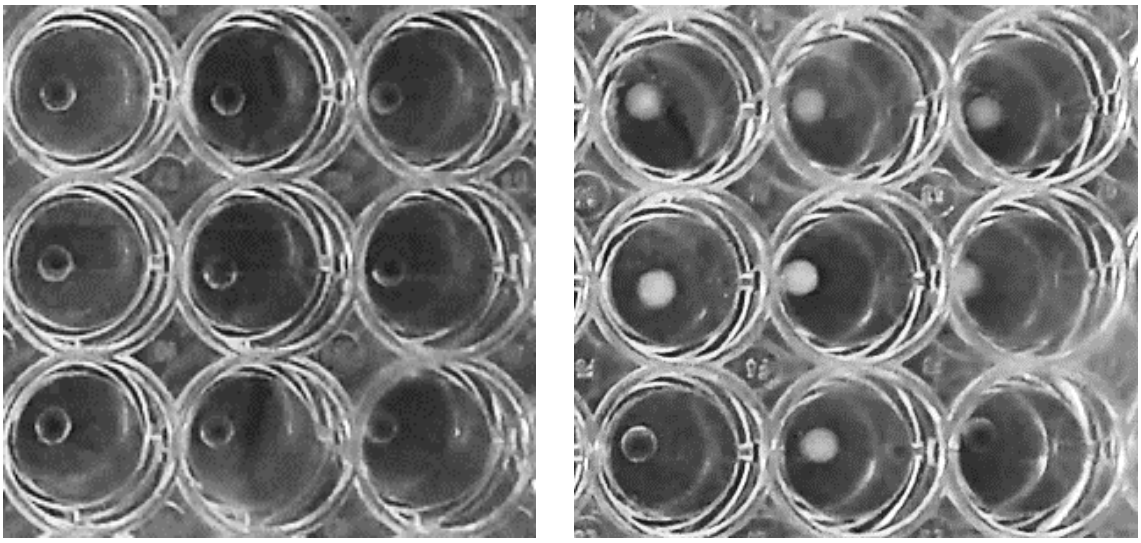

Fig. 2.2—Unfrozen (on the left) vs. frozen droplets (on the right) as recorded by the camera in the experiment.

samples, thereby causing a bias in the experiment. Individually covered wells are utilized

to keep droplets in isolation from each other and prevent this process[19].

The base of the wells are glass slides (Hampton Research; HR3-217), with a hydrophobic coating (contact angle = ~100^{o} with water) so that the droplets have a shape as close to a sphere as possible, as well as maintaining minimal contact with the slide—both of which can cause a bias in freezing experiments if incorrect. A camera continuously records video of the droplets; freezing times are later matched with the temperatures recorded on the plate. Two comparison images of liquid versus frozen droplets are in Figure 2.2 to provide an example of how these phases are differentiated during analysis.

Instrument Calibration and Temperature Validation

Calibration Experiments

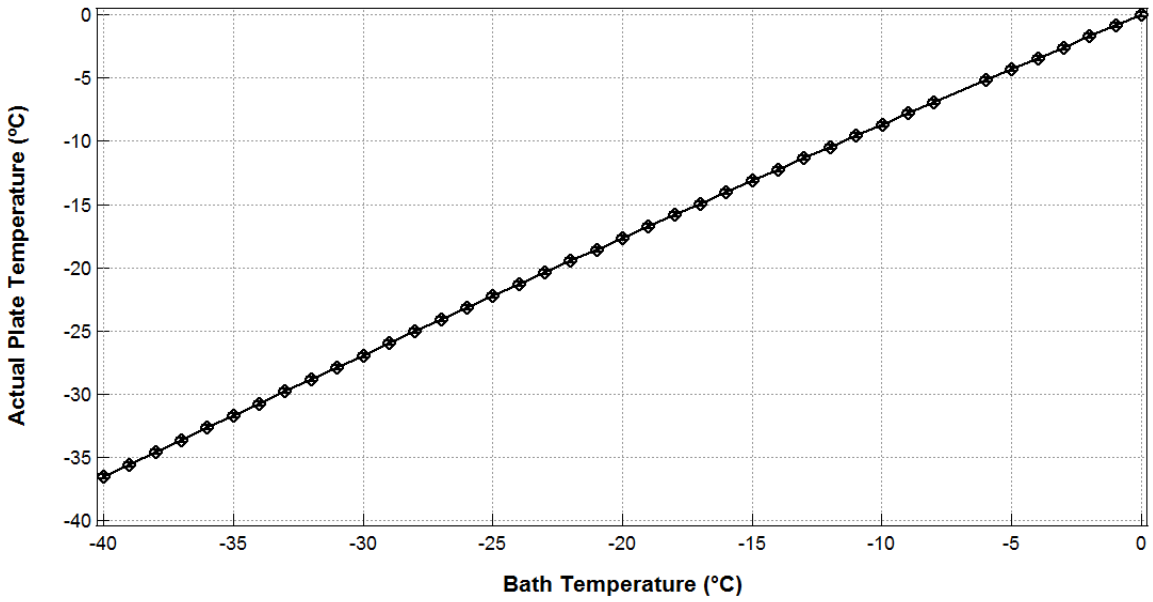

Fig. 2.3—GT-DFA plate temperature calibrations for temperature corrections in IN experiments.

Given the potential for heat loss from the plate to the room, etc., temperature validation experiments were run to confirm accurate temperature measurements for the plate during

experiments. A thermocouple (OMEGA, SA2F-K) was attached to a glass slide, and temperatures were recorded down to a bath temperature of -40°C on various locations on the plate to generate an accurate temperature profile for the DFA. Temperature differences across the plate were random and negligible, and were therefore considered to fit within the margin of error of the instrument and environmental fluctuations outside of the plate. Figure 2.3 shows the results from the temperature calibration experiments. Due to heat loss from the plate to the environment, there is a slight deviation on the plate from the bath temperature, with an increase in this difference as the temperature approaches the bath limit of -40°C. These differences have been taken into account in all following nucleation experiments.

Validation Experiments

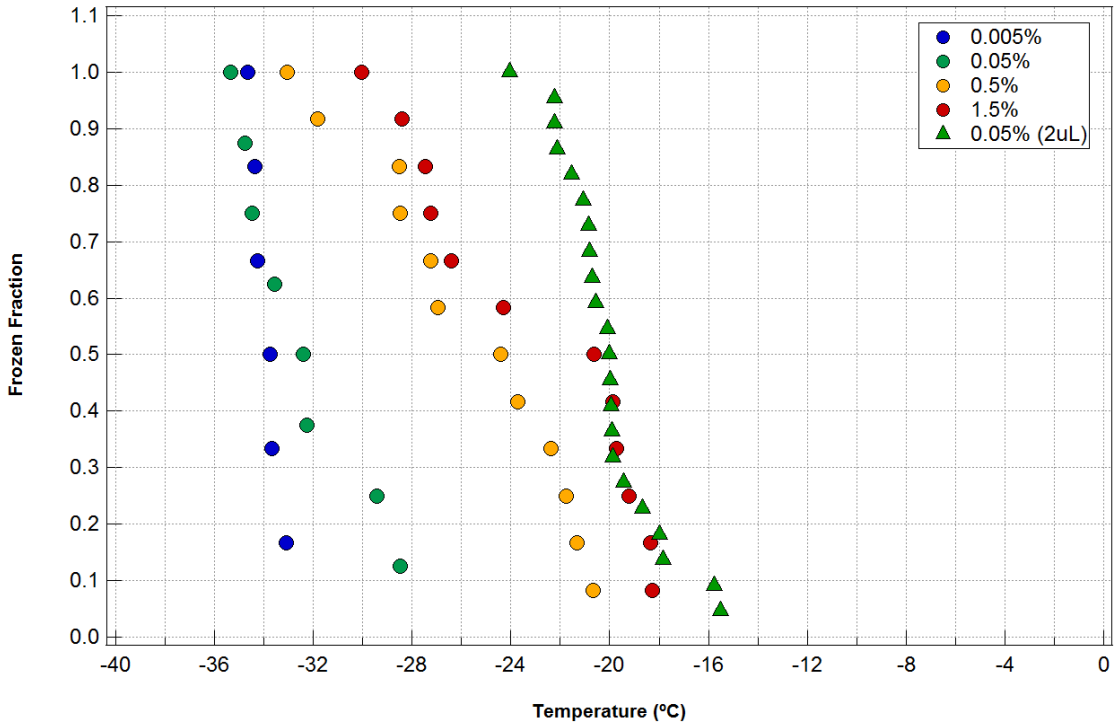

Fig. 2.4—IN activity of 1µL droplets with different weight percentages of ATD. A comparison to the 2µL droplet with 0.05% weight ATD is also included—although it should be noted that this data set comes from an experiment with a slower cooling rate and therefore is likely at a higher range of temperatures than it's counterpart would be at that volume.

To ensure the GT-DFA results are not biased by contamination, etc., validation experiments with ATD (at various weight concentrations) in milliQ water were run to compare to previous DFA studies. Results can be seen in Figure 2.4. As ATD concentration decreases within the 1μL droplets, so does their IN activity. At a low enough concentration, the behavior begins to approach homogenous freezing behavior. At a volume of 2μL, droplets began freezing around -15.5°C. It should be noted though, that the 2μl experiments were carried out congruently with the bacterial tests mentioned in Chapter 3, and therefore were tested using a different cooling rate (0.25°C/min as opposed to 1.0°C/min) which would have allowed for further stochastic freezing and higher onset freezing temperatures. Therefore, the results for the 2μl droplets can be treated as a higher limit of nucleation temperatures for this size of droplet at this weight percent of ATD. Onset nucleation temperatures from other studies compared to those shown in this thesis are given in Table 1.

Table 1—Comparison of onset nucleation temperatures of ATD in other experiments as compared to with the GT-DFA. Conditions that may have affected results in any way are included in the "Conditions" column.

Study	**Onset Nucleation Temp***	**Conditions**
Welti, et al. 2009	250-240K	Varying diameters/SA for dust particles
Kanji and Abbatt, 2010	250-235K	D_p = 100nm
Marcolli, et al. 2007	250-245K	Varying weight percents of dust
Kanji, et al. 2011	255-235K	D_p > 5μm
Koehler, et al 2010	250-235K	Varying diameters/SA for dust particles
This Study**	256-240K	Varying weight percents of dust

*Onset Nucleation Temperature is the temperature at which 1% of the total number of droplets have frozen
**Only including the 1μL droplets for consistency with the other studies

CHAPTER 3

RESULTS

Measuring the CCN activity of Biological Materials

CCN Methods

Rainwater samples were collected at various points throughout the 2015 year on the Ford ES&T building at Georgia Tech. If visible colonies were present after 10 days of growing on R2A agar plates, they were selected as potential isolates for testing. Isolates were used to make liquid stock, which were held at -80°C with glycerol until samples were ready for use [Waters et al, unpublished]. The isolates used in the CCN and IN experiments are listed in Table 2. Final cell washes from the isolates prepped for CCN experiments were also kept to use as a negative control in those experiments.

Table 2—Bacterial isolates tested in both IN and CCN experiments with their respective information.

Isolate	Name	Diameter (nm)*	Contact Angle **	Lyophilized?
9A	Alphaproteobacteria	310 – 736.5	30	No
9B	(ASK SAM)	(ASK SAM)	(ASK SAM)	Yes
11A	Massilia spp.	661.2 – 736.5	60	No
15A	Williamsia spp.	637.8 – 736.5	32?	Yes
26A	Chryseobacterium sp.	736.5 – 820.5	53?	No

*Size ranges chosen based on highest concentration detected by the CPC and confirmed with fluorescent microscopy
**Contact angles were measured using a KSV CAM-200 goniometer.

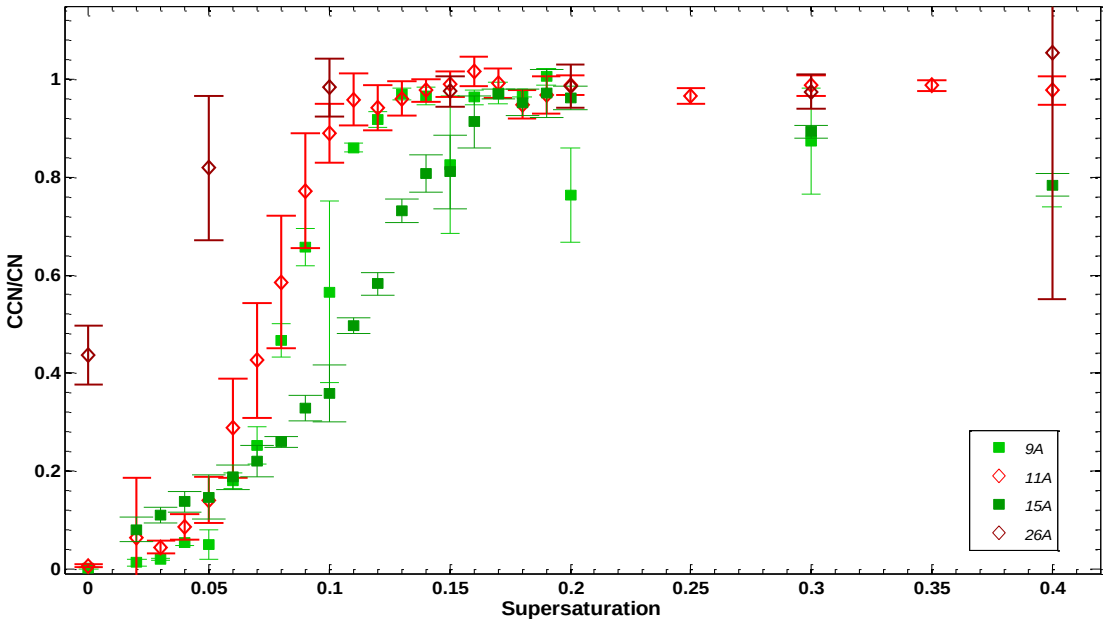

Fig. 3.1—CCN activity of the isolates tested for IN activity. Isolate 26A is supermicron which is why it appears to nucleate before supersaturation occurs (it is already visible to the OPC within the CCNc). [Waters et al, unpublished]

CCN Results

Results of the CCN experiments can be seen in Figure 3.1 [Waters et al, unpublished]. The most notable part of the graph is the data for supermicron bacteria (26A); these cells were large enough in size (i.e. larger than 0.75μm in diameter) that they are visible to the OPC of the CCN counter (CCNc) before nucleation even occurs (hence, the appearance of the bacteria nucleating before 0% supersaturation). Within the submicron bacteria data (with sizes ranging between 300 and 700nm, see Table 2), all isolates nucleated by 0.17% supersaturation, making them all very efficient CCN. For comparison, ammonium sulfate has an SS_{50} of approximately 0.6%, and dust has been shown to have an SS_{50} above at least 0.2%[51, 52]. The SS_{50}'s for the isolates in these experiments on the other hand, range between 0.06 and 0.1% SS, far below these other, and generally considered to be more important, CCN. While on its own, this has

important implications to cloud formation, it is especially important to the following IN experiments, as it shows these isolates can act as CCN well before freezing and can therefore freeze through immersion mode.

Measuring the IN activity of Biological Materials

IN Methods

Those isolates shown in Figure 3.1 were next tested for their IN activity. Droplets of varying volumes (2μL to 20μL) were pipetted onto hydrophobic glass slides, covered by a bottomless well plate as discussed in Chapter 2.1. The well plate was then placed on the cold plate in the DFA chamber, which was purged with dry air from two diffusional driers (RH ~ 5%) for approximately one hour before cooling of the plate occurred. Droplets were cooled at a rate of 0.25^{o}C/min, and the temperature was held every five degrees for three minutes to allow for stochastic freezing. Control experiments were also conducted using milliQ water, R2A media, and ATD to check the validity of all bacterial experiments. The following sections discuss various attributes of these experiments.

IN Results

Demonstrating volume/surface area dependencies

To show the importance of droplet volume used in studies, experiments were run using milliQ water and R2A media as negative controls, and ATD (0.05% WT) as a reference point for comparison to past studies. Volumes of 2, 5, 10, and 20uL were used for each sample. Figure 3.2 shows the visual difference and the reason for the potential differences these volumes can cause. The 5 and 10μL droplets see a large decrease in contact angle from the 2μL droplet, and while the 15 and 20μL droplets see a slight

Fig. 3.2—Side and top profiles of the different volumes used in the IN experiments. Increasing from right to left, the droplets are 2, 5, 10, 15, and 20L in size. Approximate contact angles for each measured by a KSV CAM-200 goniometer are included as well.

increase in that measure, they have also clearly expanded outwards, causing more contact with the surface of the plate, and thereby increasing the chance of nucleation due to contact with a surface.

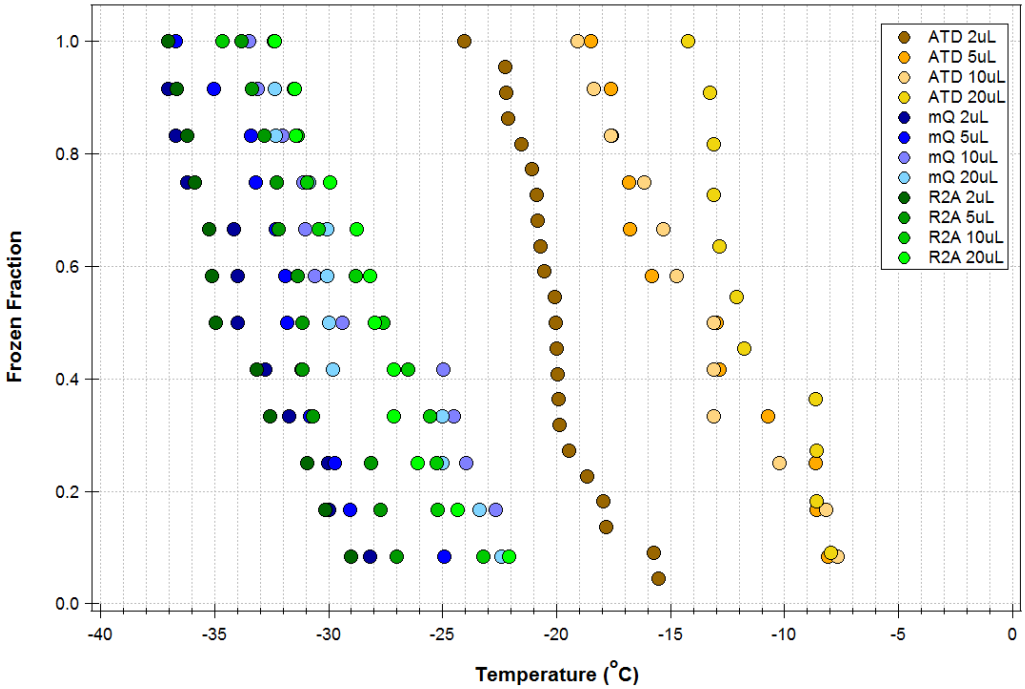

Fig. 3.3—Results from IN control experiments. MilliQ water, R2A media, and ATD are all included for comparison to bacterial experiments, as well as a demonstration of the innate volume dependence of ice nucleation.

Results of the freezing experiments with different volumes using the three aforementioned materials can also be seen in Figure 3.3, with milliQ water in blue, R2A media in green, and ATD in orange. As can clearly be seen, as volume of the droplet increases, so does the temperature at which nucleation occurs. This is supported as well, by equations (1) and (7) from Chapter 1.3 and Appendix A, respectively.

Effect of using dried out cells (i.e. Snowmax) vs. ambient samples

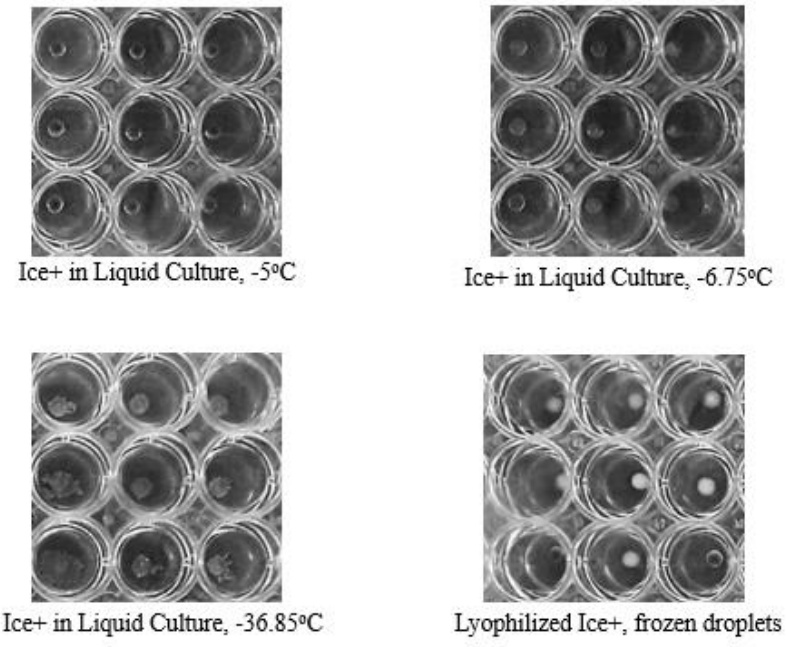

Fig. 3.4—Comparison of droplets with regular ice positive cells at various temperatures up to the homogenous limit, versus frozen, lyophilized cells.

The first experiments with an ice positive strain of Pseudomonas—which based on literature results, should freeze at temperatures above -10°C[8, 22, 53, 54]—showed no indication of freezing, even when the plate reached temperatures approaching the homogenous freezing limit. Figure 3.4 shows images of these experiments compared to actual frozen droplets. Given the pre-treatment of Snomax by lyophilizing the cells, and the large volumes used by studies utilizing untreated cells (as compared to the 2µL used in these experiments), the Pseudomonas cells were then lyophilized before being re-suspended in milliQ water and tested again. After being lyophilized, the 2µL droplets

containing these cells exhibited behavior similar to studies utilizing Snomax with similarly sized droplets[19, 55], which can be seen in Figure 3.5.

Effect of freezing in media versus milliQ water

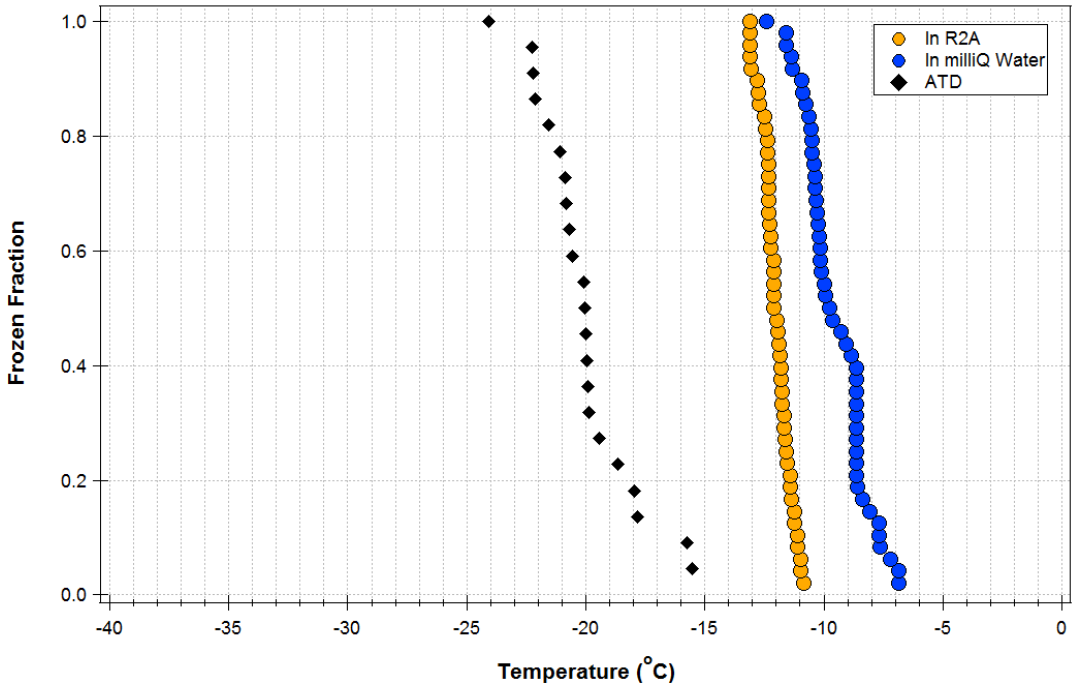

Fig. 3.5—IN activity of lyophilized ice positive bacteria, with ATD for comparison.

Experiments were also run to test the effects on the IN activity of bacteria suspended in milliQ water versus R2A media, as some studies have used the latter[56, 57]. There was a slight difference in freezing behavior, which can be seen in Figures 3.3 through 3.6. Other than isolates 9b and 15a—in which onset nucleation began at approximately the same temperatures—samples in milliQ water began freezing before those in media; furthermore, samples in milliQ water froze fully at temperatures higher than those in media, all of which indicates a clear bias in results will be present if media is used as the substrate for freezing in these experiments.

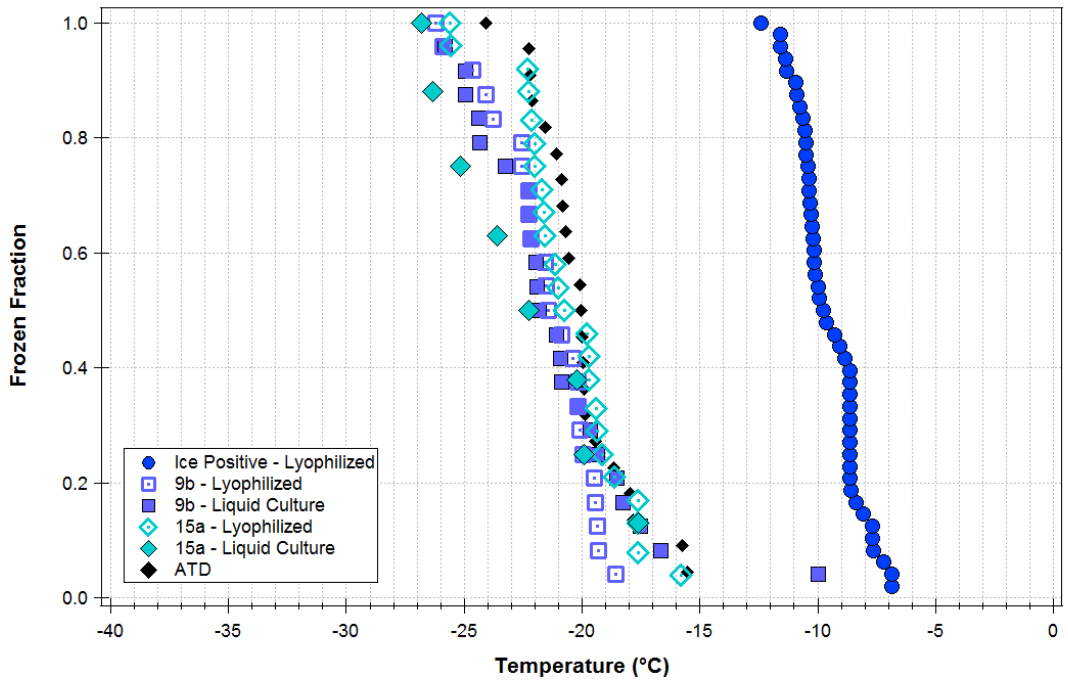

Fig. 3.6—Isolates 9b and 15a tested for IN activity with different preparations, including lyophilization, suspension in milliQ water, and suspension in R2A media. ATD and the lyophilized ice positive sample are included for reference.

Rain Isolate Results

Finally, results for each of the bacterial isolates are presented in Figure 3.7, with ATD and lyophilized, ice positive Pseudomonas included for comparison. It is important to note that each of the four isolates falls along a similar temperature range for T_{50} as well as onset nucleation temperatures. These results imply, pending further testing, that many different types of bacteria may be able to be treated as one variable within models. Furthermore, it shows that bacteria other than those that express the inaZ gene can nucleate ice at temperatures as efficiently as dust, which implies that in given circumstances, bacteria can play as large of a role in ice formation in the atmosphere as dust.

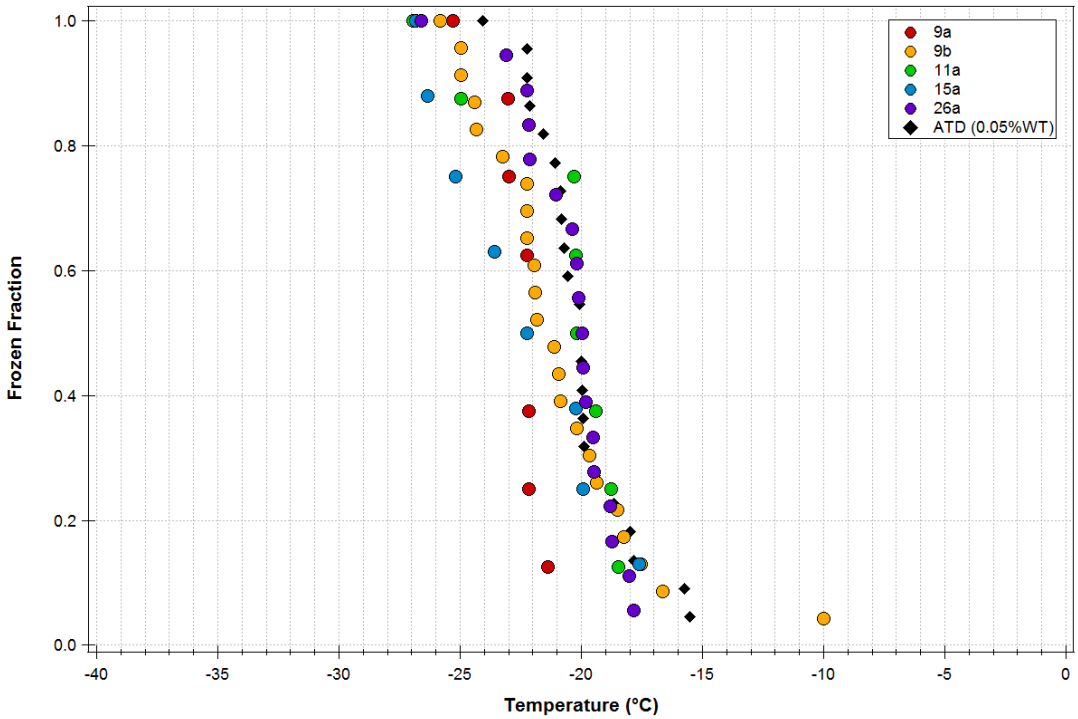

Fig. 3.7—All tested rain isolates, suspended in milliQ water. ATD is included for comparison.

CHAPTER 4

CONCLUSIONS AND FUTURE WORK

The GT-DFA is a simple but effective tool to measure immersion freezing of different aerosols. The results obtained for both milliQ water and ATD show the instrument produces data that is consistent with the literature as well as other instruments of its kind. After controlling for several variables, we were able to demonstrate the importance of consistency of methods between studies when using a DFA or similar instrument. Most importantly, volume dependence was shown within freezing results, regardless of the material (or lack thereof in the case of homogenous freezing), which is consistent with theory and past studies such as Vali 1971 and Whale, et al., 2015.

Besides the need for applying realistic cloud conditions (where even 1-2μL droplets are stretching the upper limit of cloud droplet size) to IN experiments, the results in this thesis clearly demonstrate that studies using excessive volumes, or place their samples in or on anything other than a surface that creates minimal contact with the droplets, will have biased results with temperatures higher than should be seen otherwise. To a slightly lesser degree, it was shown that the substrate used to freeze IN material also affects freezing results; samples frozen in R2A media were shown to have a positive temperature bias compared to those in milliQ water. This is somewhat expected given the composition of the media, which includes sugars, starches, amino acids, etc. Regardless, it is important to note given the experiments that have neglected to suspend their samples in milliQ water.

More notably, because of the biases created by the methods mentioned above, and on the other hand, the use of already lyophilized ice positive bacteria (i.e. Snomax) by many studies, the assumption that all ice positive bacteria (those that express the inaZ gene) nucleate ice at high temperatures is likely false. It has been seen in previours

studies too, that Pseudomonas bacteria may not cause the high level of ice nucleation, or any nucleation at all, within the homogenous limit. Ahern et al., 2007, found that while a high level of biosurfactants were produced by their Pseudomonas cells, ice nucleation did not occur. This study also used 2μL droplets, avoiding volume dependent biases within their experiment that may have caused nucleation to occur in other studies. This data and the Ahern et al, 2007, study show that more care must be taken when both making assumptions about the IN activity of Pseudomonads, as well as how samples are prepared and tested for IN activity.

Furthermore, there is sometimes an assertion made that the only relevant bacterial IN are those that express the inaZ gene; this is disproved in the results in figures 3.6 and 3.7. Not only does an ice positive, inaZ expressing bacteria not freeze above the homogenous limit without lyophilization, but all five isolates tested show IN activity on par with ATD at similar concentrations. While further experiments will have to be conducted to confirm that this holds for a larger sample of bacterial isolates, if it can be assumed that the majority of bacteria in the atmosphere exhibit the same IN activity, and that this is similar to that of dust, then the assumption that dust is the most abundant (and therefore most important) IN will no longer be a given, as concentrations of bacteria can be summed together and considered as one entity in the atmosphere and in models. Further experiments on a wide variety of isolates, with varying characteristics (size, surface activity, etc.), will need to be continued in order to confirm this hypothesis, but the results in figure 3.7 suggest that at least large varieties of bacteria can be treated as one whole within global considerations and models.

APPENDIX A

EQUATIONS AND DERIVATIONS FOR NUCLEATION THEORIES

The homogenous nucleation rate, J_{hom}, is described as the freezing rate as a function of time, divided by the total volume of the sample being tested[4, 9, 11]. This is shown in equation (3):

$$\boldsymbol{J_{hom} = \frac{R(t)}{V}} \tag{3}$$

where R(t) is equal to:

$$\boldsymbol{R(t) = -\frac{1}{N_T - N_f}\frac{dN_f}{dt} = -\frac{1}{1-f}\frac{df}{dt}} \tag{4}$$

Taking the integral of equation (4), we can solve for an equation to substitute into equation (3):

$$\boldsymbol{R(t) = -\ln(1-f)\cdot \Delta t} \tag{5}$$

So that combining equations (3) and (5) gives us the equation for the fraction of droplets frozen at a given amount of elapsed time:

$$\boldsymbol{f_{ice} = 1 - \exp(-J_{hom} \cdot V \cdot \Delta t)}$$

Which is the same as equation (1) in chapter 1.2.1. If, instead, we assume we are dealing with heterogeneous nucleation, rather than homogenous, we can replace the variable for volume, V, with the total surface area of the sample being tested. This accounts for the increase in potential freezing events due to the relative number of sites present on a given surface area of material, and gives us equation (2) from chapter 1.2.1.

To derive the singular model, we begin with the differential nucleus spectrum, k(T), defined in Vali 1971, as:

$$\boldsymbol{k(T) = \frac{1}{V}\frac{1}{N(T)}\frac{dN}{dT}} \tag{6}$$

and k(T) describes the number of nuclei in a given volume V, at a specific temperature T. If this is substituted into equation (4), but with a dependence on temperature (i.e. R(T) rather than R(t)), we end up with equation 2.

REFERENCES

1. Welti, A., et al., *Influence of particle size on the ice nucleating ability of mineral dusts.* Atmospheric Chemistry and Physics, 2009. **9**(18): p. 6705-6715.
2. Kanji, Z.A. and J.P.D. Abbatt, *The University of Toronto Continuous Flow Diffusion Chamber (UT-CFDC): A Simple Design for Ice Nucleation Studies.* Aerosol Science and Technology, 2009. **43**(7): p. 730-738.
3. Marcolli, C., et al., *Efficiency of immersion mode ice nucleation on surrogates of mineral dust.* Atmospheric Chemistry and Physics, 2007. **7**(19): p. 5081-5091.
4. Pruppacher, H.R., and James D. Klett, *Microphysics of Clouds and Precipitation*. 2 ed. 2010, New York: Springer.
5. Boucher, O. and e. al., *Clouds and Aerosols.* IPPC, 2013. **AR5**(Climate Change 2013: The Physical Science Basis).
6. Kanji, Z.A., et al., *Results from the University of Toronto continuous flow diffusion chamber at ICIS 2007: instrument intercomparison and ice onsets for different aerosol types.* Atmospheric Chemistry and Physics, 2011. **11**(1): p. 31-41.
7. Myhre, G. and e. al., *Anthropogenic and Natural Radiative Forcing.* IPPC, 2013. **AR5**(Climate Change 2013: The Physical Science Basis).
8. Hoose, C. and O. Mohler, *Heterogeneous ice nucleation on atmospheric aerosols: a review of results from laboratory experiments.* Atmospheric Chemistry and Physics, 2012. **12**(20): p. 9817-9854.
9. Murray, B.J., et al., *Ice nucleation by particles immersed in supercooled cloud droplets.* Chemical Society Reviews, 2012. **41**(19): p. 6519-6554.
10. Koehler, K.A., et al., *Laboratory investigations of the impact of mineral dust aerosol on cold cloud formation.* Atmospheric Chemistry and Physics, 2010. **10**(23): p. 11955-11968.
11. Vali, G., *Interpretation of freezing nucleation experiments: singular and stochastic; sites and surfaces.* Atmospheric Chemistry and Physics, 2014. **14**(11): p. 5271-5294.
12. Niedermeier, D., et al., *A computationally efficient description of heterogeneous freezing: A simplified version of the Soccer ballmodel.* Geophysical Research Letters, 2014. **41**(2): p. 736-741.
13. Broadley, S.L., et al., *Immersion mode heterogeneous ice nucleation by an illite rich powder representative of atmospheric mineral dust.* Atmospheric Chemistry and Physics, 2012. **12**(1): p. 287-307.
14. Whale, T.F., et al., *A technique for quantifying heterogeneous ice nucleation in microlitre supercooled water droplets.* Atmospheric Measurement Techniques, 2015. **8**(6): p. 2437-2447.
15. Cziczo, D.J., et al., *Clarifying the Dominant Sources and Mechanisms of Cirrus Cloud Formation.* Science, 2013. **340**(6138): p. 1320-1324.
16. Fitzgerald, E., et al., *Comparison of the mixing state of long-range transported Asian and African mineral dust.* Atmospheric Environment, 2015. **115**: p. 19-25.

17. Despres, V.R., et al., *Primary biological aerosol particles in the atmosphere: a review.* Tellus Series B-Chemical and Physical Meteorology, 2012. **64**.
18. Welti, A., et al., *Time dependence of immersion freezing: an experimental study on size selected kaolinite particles.* Atmospheric Chemistry and Physics, 2012. **12**(20): p. 9893-9907.
19. Budke, C. and T. Koop, *BINARY: an optical freezing array for assessing temperature and time dependence of heterogeneous ice nucleation.* Atmospheric Measurement Techniques, 2015. **8**(2): p. 689-703.
20. *Product-Snomax*. 2015 [cited 2016; Available from: www.snomax.com/product/snomax.html.
21. Lindemann, J., et al., *Plants as Sources of Airborne Bacteria, Including Ice Nucleation-Active Bacteria.* Applied and Environmental Microbiology, 1982. **44**(5): p. 1059-1063.
22. Joly, M., et al., *Ice nucleation activity of bacteria isolated from cloud water.* Atmospheric Environment, 2013. **70**: p. 392-400.
23. Lindow, S.E., et al., *Localization of Ice Nucleation Activity and the Icec Gene-Product in Pseudomonas-Syringae and Escherichia-Coli.* Molecular Plant-Microbe Interactions, 1989. **2**(5): p. 262-272.
24. Michaud, A.B., et al., *Biological ice nucleation initiates hailstone formation.* Journal of Geophysical Research-Atmospheres, 2014. **119**(21): p. 12186-12197.
25. Christner, B.C., et al., *Ubiquity of biological ice nucleators in snowfall.* Science, 2008. **319**(5867): p. 1214-1214.
26. Croteau, T., A.K. Bertram, and G.N. Patey, *Adsorption and Structure of Water on Kaolinite Surfaces: Possible Insight into Ice Nucleation from Grand Canonical Monte Carlo Calculations.* Journal of Physical Chemistry A, 2008. **112**(43): p. 10708-10712.
27. Kulkarni, G., et al., *Ice nucleation of bare and sulfuric acid-coated mineral dust particles and implication for cloud properties.* Journal of Geophysical Research-Atmospheres, 2014. **119**(16): p. 9993-10011.
28. Yakobi-Hancock, J.D., L.A. Ladino, and J.P.D. Abbatt, *Feldspar minerals as efficient deposition ice nuclei.* Atmospheric Chemistry and Physics, 2013. **13**(22): p. 11175-11185.
29. Zolles, T., et al., *Identification of Ice Nucleation Active Sites on Feldspar Dust Particles.* Journal of Physical Chemistry A, 2015. **119**(11): p. 2692-2700.
30. O'Sullivan, D., et al., *Ice nucleation by fertile soil dusts: relative importance of mineral and biogenic components.* Atmospheric Chemistry and Physics, 2014. **14**(4): p. 1853-1867.
31. Tobo, Y., et al., *Organic matter matters for ice nuclei of agricultural soil origin.* Atmospheric Chemistry and Physics, 2014. **14**(16): p. 8521-8531.
32. O'Sullivan, D., et al., *The relevance of nanoscale biological fragments for ice nucleation in clouds.* Scientific Reports, 2015. **5**.
33. Conen, F., et al., *Biological residues define the ice nucleation properties of soil dust.* Atmospheric Chemistry and Physics, 2011. **11**(18): p. 9643-9648.
34. Ginoux, P., et al., *Global-Scale Attribution of Anthropogenic and Natural Dust Sources and Their Emission Rates Based on Modis Deep Blue Aerosol Products.* Reviews of Geophysics, 2012. **50**.

35. Kirtman, B. and e. al., *Near-term Climate Change: Projections and Predictability.* IPPC, 2013. **AR5**(Climate Change 2013: The Physical Science Basis).
36. Pummer, B.G., et al., *Suspendable macromolecules are responsible for ice nucleation activity of birch and conifer pollen.* Atmospheric Chemistry and Physics, 2012. **12**(5): p. 2541-2550.
37. von Blohn, N., et al., *The ice nucleating ability of pollen: Part III: New laboratory studies in immersion and contact freezing modes including more pollen types.* Atmospheric Research, 2005. **78**(3-4): p. 182-189.
38. Diehl, K., et al., *The ice nucleating ability of pollen: Part II. Laboratory studies in immersion and contact freezing modes.* Atmospheric Research, 2002. **61**(2): p. 125-133.
39. Mohler, O., et al., *Experimental investigation of homogeneous freezing of sulphuric acid particles in the aerosol chamber AIDA.* Atmospheric Chemistry and Physics, 2003. **3**: p. 211-223.
40. Castellano, N.E., et al., *The growth of ice particles in a mixed phase environment based on laboratory observations.* Atmospheric Research, 2014. **150**: p. 12-20.
41. Diehl, K., et al., *Particle surface area dependence of mineral dust in immersion freezing mode: investigations with freely suspended drops in an acoustic levitator and a vertical wind tunnel.* Atmospheric Chemistry and Physics, 2014. **14**(22): p. 12343-12355.
42. Vali, G. and E.J. Stansbury, *Time-Dependent Characteristics of Heterogeneous Nucleation of Ice.* Canadian Journal of Physics, 1966. **44**(3): p. 477-+.
43. Stopelli, E., et al., *Freezing nucleation apparatus puts new slant on study of biological ice nucleators in precipitation.* Atmospheric Measurement Techniques, 2014. **7**(1): p. 129-134.
44. Conen, F., E. Stopelli, and L. Zimmermann, *Clues that decaying leaves enrich Arctic air with ice nucleating particles.* Atmospheric Environment, 2016. **129**: p. 91-94.
45. Joly, M., et al., *Quantification of ice nuclei active at near 0 degrees C temperatures in low-altitude clouds at the Puy de Dome atmospheric station.* Atmospheric Chemistry and Physics, 2014. **14**(15): p. 8185-8195.
46. Pinti, V., et al., *Ice nucleation efficiency of clay minerals in the immersion mode.* Atmospheric Chemistry and Physics, 2012. **12**(13): p. 5859-5878.
47. Wright, T.P. and M.D. Petters, *The role of time in heterogeneous freezing nucleation.* Journal of Geophysical Research-Atmospheres, 2013. **118**(9): p. 3731-3743.
48. Atkinson, J.D., et al., *The importance of feldspar for ice nucleation by mineral dust in mixed-phase clouds.* Nature, 2013. **498**(7454): p. 355-358.
49. Bartling, K., A. Sambanis, and R.W. Rousseau, *Multiwell microbatch crystallization on a thermal gradient.* Crystal Growth & Design, 2005. **5**(4): p. 1559-1564.
50. Wallace, J.M. and P.V. Hobbs, *Atmospheric science : an introductory survey*. 2nd ed. International geophysics series. 2006, Amsterdam ; Boston: Elsevier Academic Press. xvi, 483 p.

51. Kumar, P., I.N. Sokolik, and A. Nenes, *Measurements of cloud condensation nuclei activity and droplet activation kinetics of fresh unprocessed regional dust samples and minerals.* Atmospheric Chemistry and Physics, 2011. **11**(7): p. 3527-3541.
52. Kumar, P., A. Nenes, and I.N. Sokolik, *Importance of adsorption for CCN activity and hygroscopic properties of mineral dust aerosol.* Geophysical Research Letters, 2009. **36**.
53. Mortazavi, R., C.T. Hayes, and P.A. Ariya, *Ice nucleation activity of bacteria isolated from snow compared with organic and inorganic substrates.* Environmental Chemistry, 2008. **5**(6): p. 373-381.
54. Maki, L.R., et al., *Ice Nucleation Induced by Pseudomonas-Syringae.* Applied Microbiology, 1974. **28**(3): p. 456-459.
55. Wex, H., et al., *Intercomparing different devices for the investigation of ice nucleating particles using Snomax (R) as test substance.* Atmospheric Chemistry and Physics, 2015. **15**(3): p. 1463-1485.
56. Michigami, Y., et al., *Formation of Ice Nucleation-Active Vesicles in Erwinia-Uredovora at Low-Temperature and Transport of Inau Molecules into Shed Vesicles.* Bioscience Biotechnology and Biochemistry, 1995. **59**(10): p. 1996-1998.
57. Santl-Temkiv, T., et al., *Characterization of airborne ice-nucleation-active bacteria and bacterial fragments.* Atmospheric Environment, 2015. **109**: p. 105-117.
58. Vali, G., *Quantitative Evaluation of Experimental Results on Heterogeneous Freezing Nucleation of Supercooled Liquids.* Journal of the Atmospheric Sciences, 1971. **28**(3): p. 402-&.
59. Ahern, H.E., et al., *Fluorescent pseudomonads isolated from Hebridean cloud and rain water produce biosurfactants but do not cause ice nucleation.* Biogeosciences, 2007. **4**(1): p. 115-124.