The Importance of Biological Particles to the Ice Nucleating Particle Concentration in a Coastal Tropical Site

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Abstract. Atmospheric aerosol particles that can nucleate ice are referred to as ice nucleating particles (INP). Recent studies have confirmed that aerosol particles emitted by mid- and high-latitude oceans can act as INPs. This very relevant information can be included in climate and weather models to predict the formation of ice in clouds, given that most of them do not consider the oceans as a source of INPs. Very few studies to sample INPs have been carried out in tropical latitudes, and there is a need to evaluate their availability to understand the potential role that marine aerosol may play in the hydrological cycle of tropical regions.

This study presents results from the first measurements obtained during a field campaign conducted in the tropical village of Sisal, located on the coast of the Gulf of Mexico of the Yucatan peninsula in Mexico in January-February 2017, and one of the few data sets currently available at similar latitudes. Aerosol particles sampled in Sisal are shown to be very efficient INPs, with onset freezing temperatures as high as -3°C (in some cases), similar to the onset temperature for Pseudomonas syringae. The results show that the INP concentration in Sisal is higher than at other locations sampled with the same type of INP counter. Air masses arriving in Sisal during the passage of cold fronts have, surprisingly, higher INP concentrations than the campaign-average, despite their lower total aerosol concentration.

Biological particles were likely found to be very important in ice cloud formation at this tropical...
location, given the large concentration of INPs above -12°C. A variety of bacteria and fungi were identified. Although the majority are of terrestrial origin, some of them are clearly oceanic.

1 Introduction

Clouds are essential to the hydrological cycle of the planet and also play a significant role in the radiative balance of the climate system (Ramanathan et al., 1989; Lohmann and Feichter, 2005; Andreae and Rosenfeld, 2008; Stevens and Feingold, 2009). Cloud formation depends on the presence of cloud condensation nuclei (CCN) and most precipitation from mixed-phase clouds involves also the presence of ice nucleating particles (INPs). Aerosol-cloud interactions are one of the main sources of uncertainty in climate projections as assessed by the Intergovernmental Panel on Climate Change (Stocker et al., 2013), prompting large research efforts from the scientific community in recent years. Nevertheless, the formation and evolution of ice crystals in mixed-phase and cirrus clouds still remain highly uncertain (Seinfeld et al., 2015). Several pathways have been proposed as potentially responsible for ice formation: condensation freezing, contact freezing, immersion freezing, and deposition nucleation (Vali et al., 2015). Murray et al. (2012) and Ladino et al. (2013) have suggested that contact freezing and immersion freezing are the most efficient mechanisms leading to ice nucleation in clouds; however, the atmospheric relevance of contact freezing is still under debate (Murray et al., 2012; Kanji et al., 2017).

Most of the precipitation from deep convection in the tropics, e.g. in the Inter-tropical Convergence Zone, forms via the ice phase (Müllenstädt et al., 2015). Given the potential INP role of a variety of aerosol particles such as mineral dust, biological particles, crystalline salts, carbonaceous particles, and secondary organic aerosol, the main source of INPs at tropical latitudes is highly uncertain (Kanji et al., 2017; Yakobi-Hancock et al., 2014; DeMott et al., 2010). Although it is yet not fully understood what exactly makes an aerosol particle an efficient INP, its composition, active sites, crystal structure, size, or hygroscopicity, there is evidence that their composition is one of the key factors (Kanji et al., 2017). Mineral dust has been recognized as the most important INP on a global scale due to their good ice nucleating abilities and their elevated concentrations in the troposphere (Hoose and Möhler, 2012; Nenes et al., 2014; Atkinson et al., 2013; Kanji et al., 2017). Bioaerosol has also been identified as very efficient INP (Kanji et al., 2017; Hoose and Möhler, 2012; Fröhlich-Nowoisky et al., 2016; Hill et al., 2017) with onset freezing temperatures as high as -2 °C (Yankofsky et al., 1981; Després et al., 2012; Fröhlich-Nowoisky et al., 2015; Wex et al., 2015; Stopelli et al., 2017). Global climate models parameterize cloud droplet and ice crystal formation from observational studies and results from such modelling suggest that on a global scale bioaerosol are not a major INP source and therefore have a lower impact in ice cloud formation, in comparison to mineral dust particles (Hoose et al., 2010; Sesartic et al., 2012); however, this may not be the case on a regional scale (Burrows et al., 2013; Mason et al., 2015a). Such models also suggest that the
The most important oceanic sources of INPs are the southern oceans, north Atlantic, and north Pacific (Burrows et al., 2013; Yun and Penner, 2013; Wilson et al., 2015; Vergara-Temprado et al., 2017); however, those conclusions were drawn with little or no data from tropical latitudes.

Although important efforts were made during the 1950-70’s to understand the role of the oceans in ice cloud formation (Schnell and Vali, 1975; Schnell, 1975, 1977, 1982; Rosinski et al., 1987, 1988), recently new and robust evidence that biological material from marine environments could act as efficient INPs have been reported (Knopf et al., 2011; Wilson et al., 2015; Mason et al., 2015b; DeMott et al., 2016; Ladino et al., 2016; McCluskey et al., 2017; Irish et al., 2017; Welti et al., 2018).

Most of the past available INP data were obtained from mid- and high-latitudes studies, with tropical latitudes heavily under represented (Schnell, 1982; Rosinski et al., 1987, 1988; Boose et al., 2016; Welti et al., 2018; Price et al., 2018). Marine and coastal INP concentration ([INP]) typically ranges from $10^{-4}$ L$^{-1}$ to $10^{-1}$ L$^{-1}$ for temperatures between -10 °C and -25 °C (Kanji et al., 2017), but have shown to be higher at tropical coastal sites (Rosinski et al., 1988; Boose et al., 2016; Welti et al., 2018; Price et al., 2018). This large [INP] range may strongly depend on the microbiota concentration, the marine biological activity, and the organic matter enrichment in the sea surface microlayer as shown in Wilson et al. (2015).

At marine and coastal sites, a large variety of bacteria have been identified with *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* as the main reported phyla (Després et al., 2012). Also, airborne fungi are common in both continental and marine environments with *Cladosporium*, *Alternaria*, *Penicillium*, *Aspergillus*, and *Epicoccum* the main identified genus (Després et al., 2012). Besides bacteria and fungal spores, viruses, algae, and pollen have also been identified in the bioaerosol of marine environments (Després et al., 2012; Fröhlich-Nowoisky et al., 2015; Michaud et al., 2018). Therefore, the concentration, ice nucleating abilities, and variability of tropical bioaerosol need to be better characterized to quantify their role in cloud formation and precipitation development at regional level and within the tropical zonal band.

The Yucatan peninsula, surrounded by the Gulf of Mexico to the West and the Caribbean sea to the East, with a large variety of tropical vegetation, is a great source of both terrestrial and marine microorganisms (Guzmán, 1982; Videla et al., 2000; Morales et al., 2006). Tropical cyclones (TC) and cold fronts are some of the meteorological phenomena that seasonally affect the Yucatan peninsula every year (Whigham et al., 1991; Landsea, 2007; Knutson et al., 2010). DeLeon-Rodriguez et al. (2013) show that TC can significantly enhance the concentration of biological particles throughout the troposphere and can also efficiently transport biological particles far away from their sources. Moreover, Mayol et al. (2017) showed that ocean and terrestrial microorganisms can be efficiently transported long distances from their sources over the tropical and subtropical oceans.

This study present results of the concentration of INPs as a function of temperature and particle size, and the concentration and composition of biological particles at a tropical coastal site (Sisal, Yucatan) with the goal to infer the importance of biological particles in mixed-phase cloud formation.
and precipitation development.

2 Methods

2.1 Sampling site

Ambient aerosol particles were collected and characterized between January 21 and February 02, 2017 in the coastal village of Sisal, located in the northwest corner of the Yucatan peninsula (21°09′55″N 90°01′50″W), as shown in Figure 1. Sisal had 1837 inhabitants in 2015 (SEDESOL, 2015), with fishing and tourism being the main economical activities. The closest industry is located approximately 25 km away from the village and the nearest city is Merida, 75 km away.

The instruments used in this study were located on the roof of the Engineering Institute building of the Universidad Nacional Autonoma de Mexico (UNAM, Sisal Campus) which is 50 m from the shoreline and about 1.7 km from the center of Sisal (Figure 1). The roof is 25 m above ground level and directly faces the ocean.

January and February are part of the cold dry season in Mexico, with isolated events of rain associated with cold fronts reaching the deep tropics. The average temperature and relative humidity (RH) during the sampling period were 22.3±3.6 °C and 68.9±6.2 %, respectively.

2.2 Instrumentation

A suite of instrumentation was deployed in Sisal to characterize the aerosol chemical composition, concentration, size distribution, biological content, INPs concentration, and meteorological variables (Table 1). Most instruments were run simultaneously and next to each other (less than 10 m apart) and only wet aerosol particles were sampled. Additionally, none of the instrumentation used an impactor or cyclone prior to their inlets. The inlets were located around 1.5 m - 2.0 m above the roof surface. The meteorological data was obtained with a meteorological station (Davis, VANTAGE PRO2) placed in a different building approximately 20 m away for the other instruments.

2.2.1 Aerosol concentration and size distribution

The aerosol particle concentration and size distribution was monitored with a condensation particle counter (CPC 3010, TSI) and with an optical particle counter (LasAir II 310A, PMS), respectively. The total particle concentration reported by the CPC was collected every second at a flow rate of 1 L min⁻¹, whereas the aerosol concentration as function of size (cut-sizes at 0.3 µm, 0.5 µm, 1.0 µm, 5.0 µm, 10.0 µm, and 25 µm) was recorded every 11 s with the LasAir at a flow rate 28.3 L min⁻¹.
2.2.2 Ice nucleating particles

To determine the INP concentrations in the ambient air, aerosol particles were collected on hydrophobic glass cover slips (HR3-215; Hampton Research) with the help of a Micro-Orifice Uniform Deposit Impactor (MOUDI 110R, MSP). Identical substrate holders as those described in Mason et al. (2015a) were used to keep the glass coverslips at a location on the impaction plate where particle concentrations varied by a relatively small amount. The MOUDI has eight stages at which particles are separated and collected as a function of their aerodynamic diameter (cut-sizes are 0.18 μm, 0.32 μm, 0.56 μm, 1.0 μm, 1.8 μm, 3.2 μm, 5.6 μm, and 10.0 μm). The flow through the MOUDI is 30 L min⁻¹ and the typical sampling time was 6 h. It has been recognized that when sampling with a MOUDI under dry conditions (i.e., RH below approximately 60 %), aerosol particles can bounce from the impaction plates moving to lower stages (Winkler, 1974; Chen et al., 2011; Bateman et al., 2014). Although this is a known artifact when using this technique, this may not have been an issue in the current study given that the ambient RH was typically above 67 %. The glass substrates containing the ambient aerosol particles were stored in petri dishes at 4 °C prior to their analysis.

The INP concentrations were measured with a cold cell coupled to an optical microscope with an EC Plan-Neofluar 5 X objective (Axiolab, Zeiss) following the MOUDI-DFT method described by Mason et al. (2015a). Activation scans were conducted between 0 °C and -40 °C at a cooling rate of -10 °C per minute for particles collected on stages two to seven. Stage one was not taken into account given that the aerosol concentration on the glass substrates was typically very low, whereas in stage eight the number concentration of particles deposited on the glass substrates was too high which inhibited the proper formation of water drops.

2.2.3 Chemical composition

A second MOUDI (100NR, MSP) was operated simultaneously to collect aerosol particles for chemical composition analysis with particle sizes ranging from 0.18 μm to 10.0 μm. Particles were collected on 47 mm Teflon filters (Pall Science) for 48 h at a flow rate of 30 L min⁻¹. The filters were weighed prior and after the sampling and stored in petri dishes at 4 °C until they were analyzed. The filters were first analyzed for elemental composition and followed by ion-cation concentration analysis, performing two different analyses on each filter.

Elemental composition of the aerosol samples was determined by X-ray fluorescence (XRF), using the x-ray spectrometer at Laboratorio de Aerosoles, Instituto de Fisica, UNAM (Espinosa et al., 2012). The samples were mounted on plastic frames with no previous treatment. The analysis was carried out using an Oxford Instrument (Scotts Valley, CA, USA) x-ray tube with an Rh anode and an Amptek (Bedford, MA, USA) Silicon Drift Detector (resolution 140 eV at 5.9 keV). The tube operated at 50 kV and a current of 500 μA, irradiating during 900 s per spectrum. The efficiency of
the detection system was measured using a set of thin film standards (MicroMatter Co., Vancouver, Canada). The spectra obtained for the samples were deconvolved with the WinQXAS computer code (IAEA, 1997), and the experimental uncertainties in elemental concentrations were computed according to the method described by Espinosa et al. (2010).

After the XRF analysis, the Teflon filters were analyzed for NO$_3^-$, SO$_4^{2-}$, Cl$^-$, K$^+$, Na$^+$, Ca$^{2+}$, Mg$^{2+}$, and NH$_4^+$ using a Dionex model ICS-1500, equipped with an electrical conductivity detector, with following Chow and Watson (1999). NO$_3^-$, Cl$^-$, and SO$_4^{2-}$ were separated using a Thermo Scientific Dionex IonPac AS23-4 µm Analytical Column (4 mm x 250 mm) with Thermo Scientific Dionex CES 300 Capillary Electrolytic Suppressor module. The injection volume was 1000 µL, mobile phase was 4.5 mM Na$_2$CO$_3$ - 0.8 mM NaHCO at 1 mL min$^{-1}$ flow rate. For NH$_4^+$, Na$^+$, Ca$^{2+}$, Mg$^{2+}$, and K$^+$, volumes of 1000 µL were injected in a Thermo Scientific Dionex IonPac CS12A Cation-Exchange Column (4 mm x 250 mm) with the Thermo Scientific Dionex CES 300 Capillary Electrolytic Suppressor. The mobile phase was a solution CH$_3$SO$_3$ 20 mM and 1 mL min$^{-1}$ flow rate.

2.2.4 Biological particles

Air samples were collected using two Quick Take 30 Sample Pump BioStage viable cascade impactor (SKC Inc. USA), which is a one-stage portable battery-powered instrument operated at a constant airflow rate (28.3 L min$^{-1}$) for a sampling time of 5 min. Petri dishes containing Trypticase soy agar (TSA; BD Bioxon) media, supplemented with 100 mg L$^{-1}$ cycloheximide (Sigma-Aldrich) to prevent fungal growth, were used for capture cultivable total bacteria, and Malt extract agar (EMA; BD Bioxon) for cultivable airborne propagule fungi. The two impactors, one with the TSA and the other one with EMA growing media, were run in parallel. After exposure, the plates were incubated at 37 $^\circ$C during 24 h - 48 h for cultivable total bacteria and at 25 $^\circ$C during 48 h - 72 h for propagule fungi. After incubation, colonies growing on each plate were counted and concentrations were calculated by taking the sampling rates into account and reported as colony-forming units per cubic meter (cfu m$^{-3}$) of air. The petri dishes with the grown colonies were stored at 4 $^\circ$C prior to their analysis. Fungi were identified to genus level by macroscopic characteristics of the colonies and microscopic examination of the spore structure. Representative bacterial colonies were picked and purified using several transfer steps of single colonies on TSA and checked by Gram-staining and microscopy. Fresh biomass of the bacterial isolates were suspended in 30 % glycerol LB-broth (Alpha Biosciences, Inc.) and stored at -72 $^\circ$C, for further analyses.

Bacteria isolated from the pure cultures were identified by 16S rRNA sequencing. DNA was extracted using the QIAamp DNA Mini kit (QIAGEN), according to the manufacturers protocol. Partial 16S rRNA gene sequences were amplified by polymerase chain reaction (PCR) using universal bacterial primers 27F (5-AGA GTT TGA TCM TGG CTC AG-3) and 1492R (5-TAC GGY TAC CTT GTT ACG ACT T-3) (Lane, 1991). PCRs were performed in a total volume of 50 µL including
2 µL of bacterial DNA, 35.4 µL of ddH2O, 5 µL of 10 X buffer, 1.5 µL of MgCl2 (1.5 mM), 1 µL of dNTPs (10 mM), 0.1 µL of Taq DNA polymerase (5 U µL−1), and 2.5 µL each primer (10 µM). Cycle conditions were as follows: initial denaturation at 94 °C for 1 min; followed by 35 cycles at 94 °C for 1 min, 56 °C for 30 s, 72 °C for 1.5 min; and a final extension at 72 °C for 5 min. The PCR products were examined for size and yield using 1.0 % (w/v) agarose gels in TAE buffer. After successful amplification, the obtained products were sequenced using a PRISM 3730 automated sequencer (Applied Biosystem Inc.). DNA sequences were edited and assembled using the Seq-Man and Edit Seq software (Chromas Lite, Technely Slom Pty Ltd. USA). Sequence similarity analysis was performed using the BLAST software (http://www.ncbi.nlm.nih.gov/BLAST).

Although specific growing media for actinobacteria were not used in this study, some actinobacteria colonies were able to grow on the TSA petri dishes; therefore, in some cases they were isolated and identified as follows. Genomic DNA was extracted using standard protocols reported previously for actinobacteria (Maldonado et al., 2009). The DNA preparations were then used as template for 16S rRNA gene amplification using the universal set of bacterial primers 27f and 1525r (Lane, 1991). The following components for the PCR mix were employed: 0.5 µL DNA template (for a final concentration of 100 ng µL−1), 5 µL 10X DNA polymerase buffer, 1.5 µL MgCl2 (50 mM stock solution), 1.25 µL dNTP (10 mM stock mixture), 0.5 µL of each primer (20 µM stock solution), 2.0 units of Taq polymerase made up to 50 µL with deionized sterile distilled water.

The PCR amplification was achieved using a Techne 512 gradient machine using the protocol described in Maldonado et al. (2008). The expected product (size aprox 1,500 bp) was checked by horizontal electrophoresis (70 V, 40 min) and then purified using the QIAquick PCR purification kit (QIAGEN, Germany) following the manufacturers instructions. Purified 16S rRNA gene PCR products were sent for sequencing to Macrogen (Korea) for the DyeDeoxy Terminator Cycle Sequencing kit (Applied Byosystems). Assembly of each 16S rRNA gene sequence was performed using Chromas (www.technelysium.com.au) and checked manually with the SeaView software (Galtier et al., 1996). Each assembled sequence was compared against two databases, namely, (a) the GenBank database (www.ncbi.nlm.nih.gov) by using the BLAST option and (b) the EZCloud (www.ezbiocloud.net) under its EZTaxon option. Both databases generated a list of the closest phylogenetic neighbors to each sequence and the EZTaxon, specifically provided the list of the closest described (type) species. At least 650 bp were employed for the analyzes.

3 Results and Discussion

3.1 Aerosol Concentration and Meteorology

Figure 2 shows the time series of the aerosol particle concentration between January 21 and February 02, 2017. Note the large diurnal variability for the aerosol particle concentration measured by the CPC (particles > 30 nm) and the LasAir (particles >300 nm), with average concentrations for the
entire sampling period of 896±570 cm$^{-3}$ and 0.026±0.022 cm$^{-3}$, respectively. The data reported by the CPC and the LasAir indicate that most of the aerosol particles were smaller than 300 nm. A similar result was found by Rosinski et al. (1988) in the Gulf of Mexico (GoM) who found that the aerosol concentration for particles ranging between 0.5 µm and 1.0 µm was three to four orders of magnitude smaller than particles ranging between 0.003 µm and 0.1 µm. A decrease in aerosol particle concentration was observed at the arrival and during the passage of two cold fronts (indicated by the vertical grey areas in Fig. 2 and labeled A and B) during the sampling period. Associated with the arrival of the cold fronts, horizontal wind speed at the site increased by at least a factor of three, as seen in Figure 3. During cold front A, the wind direction was almost constant from approximately 270 °, while during the cold front B the wind direction varied between 270 ° and 360 °, with a more northerly component and a larger influence from the GoM compared to winds associated with cold front A.

Hysplit back-trajectories from the measurement site were run for 72 h for each of the days of the campaign. In the absence of cold fronts A and B, the air masses arriving in Sisal had a very likely large continental influence, associated with southerly winds (Figure S1). However, when the cold fronts A and B reached the Yucatan Peninsula, northerly and northwesterly winds prevail, associated with more marine influence. The arrival of the cold fronts was also confirmed with the National Oceanic and Atmospheric Administration (NOAA) surface maps for January 22 and 29 (Figure S2).

Air masses behind both cold fronts, flowing over the GoM, were characterized by lower aerosol particle concentrations than air masses coming from the south to the site. This result agrees well with a large body of evidence indicating that marine air masses have lower aerosol particle concentration than continentally-influenced air masses (Patterson et al., 1980; Fitzgerald, 1991). Note, however that the number size distributions of the aerosol particles larger than 300 nm did not significantly change before, during or after the passage of the cold fronts (Figure S3).

### 3.2 Ice Nucleating Particle Concentration ([INP])

A total of 41 samples (8 stages each) were collected during the Sisal field campaign to calculate the [INP] as a function of temperature and particle size. Some of these samples showed a high ice nucleating activity with high [INP] at temperatures close to 0 °C. In some cases the onset freezing temperatures were found to occur at temperatures as high as -3 °C. Figure 4 summarizes the [INP] as a function of temperature for 29 analyzed samples (bluish area with red edges). Due to technical issues it was not possible to analyze the samples collected after January 30th. The present results are superimposed on the data reported by Kanji et al. (2017) to conduct a direct comparison with previous studies.

At temperature above -12 °C the [INP] measured in Sisal was slightly higher than the values reported by DeMott et al. (2016) at the continental boundary layer in the US. Aerosol particles acting as INPs at these warm temperatures are usually recognized as biological given that other
aerosol particles such as metals, crystalline salts, combustion particles (e.g., soot), and organics are unable to nucleate ice crystals under these conditions. Moreover, for typical concentrations of mineral dust in the atmosphere, ice nucleation by mineral dust at these temperatures is not important (Hoose and Möhler, 2012; Murray et al., 2012; Kanji et al., 2017). The potential sources of the measured INPs in Sisal are discussed below.

Figure 5 is based on Mason et al. (2016) and shows the average [INP] for three different temperatures (-15 °C, -20 °C, and -25 °C) at different locations around the globe using the same sampling and analysis methods. The Sisal data corresponds to particle diameters ranging between 0.32 µm and 10 µm where 16 out of the 29 samples fulfilled the size criteria. At -15 °C the average [INP] in Sisal was lower than Kolby (USA) and Labrador Sea; however, the obtained values are comparable to those found at UBC (Canada), Saclary (France), and Ucluelet (Canada). At -20 °C and -25 °C the average [INP] in Sisal were comparable or higher than at the other locations. As shown by the stars on top of the Sisal bars, the [INP] during the passage of the cold fronts was found to be higher than the average [INP] although the obtained values are within the error bars. For example, at -15 °C the [INP] increases from 0.28 L⁻¹ to 0.46 L⁻¹ in the cold air mass after the passage of cold front B. Recalling that the air masses behind cold front B contained a lower aerosol particle concentration, this suggests that the marine particles in that air mass are more efficient INPs than in the air masses with more continental influence.

Although the majority of the field studies performed to measure the [INP] have been conducted in mid-latitudes, we compare with results for the few published studies from tropical latitudes and tropical coastal locations. Rosinski et al. (1988) measured the [INP] in the condensation freezing mode for particles in the GoM during a cruise. The study reports very efficient INPs with onset freezing temperatures as high as -4 °C for particles with diameters between 0.1 µm and 0.4 µm. On August 6, 1986 (the closest sampling site to Sisal in the GoM) the study shows that the [INP] at -15 °C was on the order of 10⁻² L⁻¹ for particles with sizes between 0.1 µm and 0.4 µm. The results from Rosinski et al. (1988) differ from the present results where the [INP] at -15 °C was found to vary between 10⁻¹ L⁻¹ and 10⁰ L⁻¹ for particles ranging between 0.32 µm and 10 µm. This discrepancy could be attributed to the differences in the size of the particles sampled. As shown by DeMott et al. (2010) particles larger than 500 nm are the more likely potential INPs and as stated by Mason et al. (2016) super-micron particles are a large contributor to the INP population. Additionally, the chemical composition of the aerosol particles collected by Rosinski et al. (1988) indicate that the air masses in the GoM in July-August were largely influenced by mineral dust particles. This is further discussed below.

3.2.1 [INP] vs. particle size

Figure 6 shows the mean [INP] concentration as a function of particle size between 0.32 µm and 10 µm at four different temperatures (-15 °C, -20 °C, -25 °C, -30 °C). The INP size distributions
were different for the temperatures considered, in contrast with the results obtained by Mason et al. (2015b) at the Pacific coast of Canada. At -15 °C the peak concentration was found to be in particles ranging between 1.0 µm and 1.8 µm. This has been reported as the typical size for airborne bacteria (Burrows et al., 2009). Similar size distributions were obtained at -20 °C and -25 °C with a peak concentration for particles ranging in size between 3.2 µm and 5.6 µm. Finally, at -30 °C the peak was observed at smaller sizes (i.e., between 1.8 µm and 3.2 µm). Figure 6 also shows that most of the INPs are in the supermicron size range, where submicron particles represent less than 10 % of the total [INP] independent of temperature in agreement with the results found by Mason et al. (2015b) and Mason et al. (2016).

### 3.3 Identification of the Potential INP Sources

The chemical analyzes of the sampled aerosol particles indicate that a large fraction of the particle mass (for sizes between 0.18 µm to 10.0 µm) are likely of marine origin (Figure 7(A-B)). Both techniques, i.e., XRF and HPLC found that the main elements/cations/ions are sodium and chlorine. The low concentrations of Ti, Cu, and Zn shows the very low probability of anthropogenic influence at the sampling site. However, although sulfate and ammonium can be emitted by natural sources, their presence, in addition to nitrates, indicate that the influence of anthropogenic activities to the aerosol population is not completely negligible. Finally, the low concentration of Al, Fe, and Si suggest that mineral dust is not a major contributor of aerosol particle mass during the sampling period. The long-range transport of mineral dust particles from Africa to the Yucatan Peninsula and the GoM is very rare between January and February, compared with the more frequent transport probability during July-August (Rosinski et al., 1988; Kishcha et al., 2014). Rosinski et al. (1988) reported that the concentration of Al, Fe, and Si in July-August 1986 in the GoM were higher than the sodium and chlorine concentrations, although the aerosol was supposed to be of marine origin.

Figure 7(C-D) show the mass size distribution of the main five elements/cations/ions determined by the XRF and HPLC techniques. For the XRF analyzes Na, Cl, and Ca, have a single peak at 3.2 µm, whereas the S and Mg reported two peaks at 0.32 µm and 3.2 µm. Similar to the XRF results, the HPLC analyzes for Na⁺ and Cl⁻ also showed a single peak at 3.2 µm. SO₄²⁻, NO₃⁻ showed two peaks at 0.32 µm and 3.2 µm, whereas for NH₄⁺ the peaks were located at 0.32 µm and 5.6 µm. The obtained size distributions are in agreement with those of sea salt type particles as reported elsewhere (O’dowd et al., 2004; Prather et al., 2013). Given the low resolution of these measurements (i.e., 48 h) we were unable to quantitatively separate the chemical composition of the aerosol particles during the passage of cold fronts to identify the composition of the efficient INPs observed.

Given that mineral dust particles are unlikely the source of the measured INPs above -12 °C, and as organics and soot are not typically efficient INPs at temperatures above -15 °C (Kanji et al., 2017), in addition to the supermicron size of ca. 90 % of the INPs, bioparticles are the best candidates as the
source of INP, as bioparticles have been shown to efficiently nucleate ice at those high temperatures (Hoose and M"ohler, 2012; Murray et al., 2012; Ladino et al., 2013). To confirm the presence of bioparticles around Sisal and to determine their potential role in the ice nucleating abilities of the collected aerosol particles, bacteria and fungi identification was performed.

Samples for viable bacteria and fungi were collected every day at 6:00, 8:00, 10:00, and 12:00 local time. However, a single daily profile was performed between January 22 and 23. Bacteria and fungi colony forming units (cfu) m\(^{-3}\) were usually above zero with the highest concentrations found early in the morning (Figure S4). The bacteria and fungi concentrations showed a relatively good correlation (r=0.55, p<0.0005 not shown) with average values for the whole sampling period of 295±312 cfu m\(^{-3}\) and 438±346 cfu m\(^{-3}\), respectively. The bacteria concentration are comparable to the values found by Hurtado et al. (2014) in Tijuana, on the Pacific coast of Mexico (i.e, 230 cfu m\(^{-3}\) - 280 cfu m\(^{-3}\)). Bacteria and fungi concentrations were found to be lower when the wind was coming from the north in comparison with southern-continental air masses (Figure S5), a behavior similar to the aerosol concentration shown in section 3.2.

Figure 8 shows the time series of the [INP] together with the bacteria and fungal concentrations. Panels B and C show a poor correlation between the bacteria and fungal concentrations with the [INP] with correlation coefficients at -15 °C of 0.12 (p=0.06) and 0.36 (p=0.03), respectively. This poor correlation can be in part due to the different timings of the MOUDI and the biosamplers which lead to uncertainties in the analysis. Another additional factor is the fact that the reported bacteria and fungi concentrations are only a small fraction of the total population given that the used method is selective to viable microorganisms only. Note that the fraction of detected microorganisms by culture methods are typically ca. 1 % (but can be lower) of the total population (Lighthart, 2000; Burrows et al., 2009). From Figure 8 it is notable that although the bacteria and fungi concentrations were very low on January 29 (i.e., under the influence of the cold front B), the [INP] at -15 °C was comparable to the average value for the entire campaign. It is therefore intriguing if the marine microorganisms brought to Sisal by the cold front B could be efficient INPs.

Table 2 summarizes the identified bacteria before the arrival of cold front A and after the passage of cold fronts A and B. Additionally, Table 3 shows the fungi identification for the whole campaign. To our knowledge this is the first time that airborne viable bacteria, and fungi are identified at this coastal location. Although biological microorganism characterization has been previously conducted in Mexico, those studies focused on health effects mainly (Santos-Burgoa et al., 1994; Guzman, 1998; Maldonado et al., 2009; Frías-De León et al., 2016; Ríos et al., 2016). Note that 76 % of the detected bacteria were Gram positive with Micrococcus, Staphylococcus, and Bacillus as the main identified genus (Figure S6). As shown in Table 2, before the arrival of cold front A (January 21-22), a large variety of bacteria species were found with different typical sources, mostly terrestrial. This is in contrast with the identified species found after the passage of cold fronts A and B. Especially, after cold front B different Vibrio species were identified, most of which are typically of marine
origin. Recently, Hurtado et al. (2014) found that the most common genera of the bacteria in Tijuana were *Staphylococcus*, *Streptococcus*, *Pseudomonas*, and *Bacillus* in close agreement with the present results. Regarding fungi, different genera were also identified as shown in Table 3 with *Cladosporium* and *Penicillium* as the most frequent ones (51% and 11%, respectively) as shown in Figure S6. This is in good agreement with the data reported by Després et al. (2012).

Several studies have shown the good correlation between the concentration of fluorescent biological particles and the [INP]; however, from those studies it is highly uncertain if the good ice nucleating abilities can be attributed to a single microorganism species (Mason et al., 2015b; Twohy et al., 2016). Off-line methods as the one used here have been able to identify from rain water and cloud water specific microorganisms such as *Pseudomonas syringae*, *Micrococcus*, *Staphylococcus*, *Cladosporium*, *Penicillium*, *Aspergillus* among others, with some showing good ice nucleating ability (Amato et al., 2007, 2017; Delort et al., 2010; Failor et al., 2017; Stopelli et al., 2017; Akila et al., 2018).

4 Conclusions

Aerosol particles around Sisal (on the northwest coast of the Yucatan peninsula) were found to be efficient INPs with onset freezing temperatures as high as -3 °C, similar to the onset freezing temperature of the well known efficient INP *Pseudomonas syringae* (Wex et al., 2015) and Arctic sea surface microlayer organic-enriched waters (Wilson et al., 2015). The results show that the INPs concentration in Sisal are comparable (in specific cases even higher) than at other locations studied using the same INP counter type, especially under the influence of cold fronts. This is an intriguing result given that the air masses behind the cold front contained lower aerosol particles concentrations. This deserves further analysis given than the Yucatan peninsula and the Caribbean region are impacted by this meteorological phenomenon during the winter and early spring months.

The chemical analyzes performed on the sampled aerosol particles did not indicate the presence of mineral dust particles in significant concentrations (the combined mass concentrations of Al, Si, and Fe correspond to 5.1% of the total particle mass measured by the XRF), not surprisingly since African dust is mainly transported to South America during January (Prospero et al., 2014). The large concentration of INPs above -15 °C, and the large size of 90% of the INPs, indicate that the likely source of the INPs measured in Sisal are biological particles. Therefore, continental and probably maritime biological particles are believed to play an important role in ice cloud formation and precipitation development in the Yucatan peninsula. Although several bacteria and fungi were identified, it is unknown if any of them were responsible for the observed ice nucleating abilities of the aerosol around Sisal. As stated by Islebe et al. (2015) both bacteria and fungi need to be properly documented in the peninsula and the GoM to fully understand their regional importance.
The quantitative understanding of the importance of biological particles in ice particle formation is a challenging task for the cloud physics community. As shown here, even when combining biology with chemistry, physics, and meteorology, the results obtained are not as quantitative as would be desired. Efficient INPs such as those measured in Sisal could be very important for cloud glaciation. Additionally, they can trigger ice multiplication or secondary ice formation at such high temperatures via the Hallett-Mossop mechanism (Hallett and Mossop, 1974; Field et al., 2017) and impact precipitation formation. Therefore, further studies are needed in order to improve our current limited understanding of the role that tropical microorganisms could play in ice cloud formation.

Author contributions. LAL and GBR designed the experiments. LAL, HAO, MAE, and BF carried out the INP and aerosol measurements. IR, LM, ES, EQ, LAM, and AGR analyzed the biological particles. HAO and JM performed the chemical analyzes. LAL, ZRD, CC, AKB, MS and VI performed the INP analyzes. LAL wrote the paper, with contributions from all co-authors.

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**Fig. 1.** Map showing the sampling location. The red star shows the location of the Engineering Institute building where the sampling took place (GoogleMaps).

**Table 1.** List of the measured variables and the corresponding instrumentation.

| Measured Variable                  | Instrument                                                                 |
|-----------------------------------|-----------------------------------------------------------------------------|
| INP concentration                 | MOUDI-DFT (Mason et al., 2015a)                                            |
| Aerosol concentration             | Condensation particle counter (CPC, TSI 3010)                               |
| Coarse aerosol size distribution  | LasAir Optical particle counter (MSP)                                       |
| Chemical composition              | X-Ray fluorescence and High-performance liquid chromatography                |
| Bacterial and fungal concentration| Biotage impactor (SKC)                                                     |
| Meteorology                       | Weather station (Davis)                                                    |
Fig. 2. Aerosol particle concentration time series for the whole campaign. A) measured by the CPC, B) measured by the LasAir full size range (0.3 \( \mu \)m to 25 \( \mu \)m), and C) measured by the LasAir for particles \( >500 \) nm (0.5 \( \mu \)m to 25 \( \mu \)m). Grey areas denote the periods affected by the entrance of a cold front (A and B). Each X-axis tick corresponds to midnight local time.

Fig. 3. A) Time series of the total aerosol particle concentration measured by the CPC, and B) Time series of the wind speed (yellow) and wind direction (green). Each X-axis tick corresponds to midnight local time.
Fig. 4. Summary of INP concentrations taken from studies of field measurements conducted globally (adapted from Kanji et al. (2017)).
Fig. 5. Mean INP number concentrations at droplet freezing temperatures of -15 °C (light gray), -20 °C (dark gray), and -25 °C (black). The blue and red stars represent the mean INP concentration during the cold fronts A+B and cold front B, respectively (adapted from Mason et al. (2016)).

Fig. 6. Mean INP concentration as a function of aerosol particle size at A) -15 °C, B) -20 °C, C) -25 °C, and D) -30 °C.
Fig. 7. A) Mean mass concentration of 14 detected elements for the collected aerosol particles using XRF, B) Mean mass concentration of 8 detected ions/cations for the collected aerosol particles using HPLC, C) and D) Mean mass size distribution of the main five detected elements/cations/ions with the XRF and HPLC, respectively.

Fig. 8. A) Time series of the [INP] at -15 °C (blue), -20 °C (brown), and -25 °C (yellow), B) Time series of the [INP] at -15 °C (blue) together with bacteria concentration (red), and C) Time series of the [INP] at -15 °C (blue) together with fungal concentration (black). Each X-axis tick corresponds to 06:00 local time. The horizontal error bars indicate the time span the MOUDI-DFT measurements i.e., 6 h. Grey areas denote the periods affected by the entrance of a cold front (A and B).
Table 2. Bacterial isolation for top) Jan 21-22, middle) Cold front A, bottom) Cold front B. a, Isolated on TSA media; b, Isolated on GYM media.

| Phylum            | Genus/species                      | Source                                                                 |
|-------------------|------------------------------------|----------------------------------------------------------------------|
| Actinobacteria    | Kocuria palustris                  | Soil, rhizoplane                                                      |
|                   | Micrococcus spp                    | Water, soil, dust, and skin                                           |
|                   | Rhodococcus corynebacteroides      | Soil, water and eukaryotic cells                                     |
| Firmicutes        | Staphylococcus kloosii             | Human and animal skin                                                |
|                   | Staphylococcus lugdunensis         | Human and animal skin                                                |
|                   | Staphylococcus nepalensis          | Mucocutaneous zones of humans and animals                            |
|                   | Staphylococcus arlettae            | Animal skin, mucosal zones, polluted water                           |
|                   | Staphylococcus epidermidis         | Human skin, mucosal microbiota                                        |
|                   | Bacillus aryabhatai                | Upper atmosphere, rhizosphere                                         |
|                   | Bacillus gibsonii                  | Alkaline soil                                                         |
|                   | Bacillus aeris                     | Soil                                                                  |
|                   | Staphylococcus lentus              | Soil                                                                  |
| Alphaproteobacteria| Sphingomonas mucosissima           | Water and soil                                                        |
| Actinobacteria    | Micrococcus spp                    | Water, soil, dust, and skin                                           |
| Firmicutes        | Bacillus oceanisediminis           | Marine sediments                                                      |
| Gammaproteobacteria| Proteus mirabilis                  | Water and soil                                                        |
|                   | Pseudomonas stutzeri               | Soil                                                                  |
| Actinobacteria    | Micrococcus spp                    | Water, soil, dust, and skin                                           |
|                   | Micrococcus lentus                 | Soil, dust, water and air                                             |
|                   | Micrococcus yunnanensis            | Roots of Polyspora axillaris                                         |
|                   | Streptomyces spp                   | Cosmopolitan                                                          |
| Firmicutes        | Bacillus spp                       | Cosmopolitan                                                          |
|                   | Bacillus niacini                   | Soil                                                                  |
|                   | Bacillus subtilis                  | Soil                                                                  |
|                   | Planomicrobium koreense            | Fermented seafood                                                     |
|                   | Staphylococcus spp                 | Human and animal skin, mucous zones, soils                           |
|                   | Solibacillus isronensis            | Air                                                                   |
|                   | Staphylococcus equorum             | Human and animal skin                                                |
| Gammaproteobacteria| Pseudomonas reactants              | Soil                                                                  |
|                   | Vibrio alginolyticus               | Marine                                                                |
|                   | Vibrio natriegens                  | Marine                                                                |
|                   | Vibrio neocaledonicus              | Marine                                                                |
|                   | Vibrio parahaemolyticus            | Marine                                                                |
|                   | Zobellella sp.                     | Marine and estuarine environments                                    |
Table 3. Fungal identification on EMA media for the whole sampling period

| Phylum              | Genus      | Source                                                                 |
|---------------------|------------|------------------------------------------------------------------------|
| Dothideomycetes     | *Alternaria* | Dead plants, soil, foods, air, indoor                                  |
|                     | *Cladoprium* |                                                                         |
|                     | *Drechslera* |                                                                         |
| Euascomycetes       | *Curvularia* | enviroments, decaying organic matter,                                    |
| Eurotiomycetes      | *Aspergillus* | bioaerosols in, on animal systems and                                    |
|                     | *Penicillium* | in freshwater and marine habitats.                                     |
| Leotiomycetes       | *Monilia*   |                                                                         |
| Sordariomycetes     | *Fusarium*  |                                                                         |
| Zygomycetes         | *Rhizopus*  |                                                                         |