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Identification and quantification of ice nucleation active microorganisms by digital droplet PCR (ddPCR)

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Several bioaerosol types, including bacteria, fungi, pollen and lichen, have been identified as sources of biological ice nucleators (IN) which induce ice formation already at temperatures as high as -10 °C or above. Accordingly, they potentially contribute widely to environmental ice nucleation in the atmosphere and are of great interest in the study of natural heterogenous ice nucleation processes. Ice nucleation active microorganisms have been found and studied among bacteria (Proteobacteria) and fungi (phyla Basidiomycota and Ascomycota). The mechanisms enabling the microorganisms to ice nucleation are subject to ongoing research. While it has been demonstrated that whole cells can act as ice nucleators in the case of bacteria due to the presence of specific membrane proteins, cell-free ice nucleation active particles seem to be responsible for this phenomenon in fungi and lichen. The identification and quantification of these ice nucleation active microorganisms and their IN in atmospheric samples is crucial to understand their contribution to the pool of atmospheric IN. This is not a trivial task since the respective microorganisms are often prevalent in lowest concentrations and a variety of states, be it viable cells, spores or cell debris from dead cells.
Molecular biology provides tools to identify and quantify ice nucleation active microorganisms independent of their state by detecting genetic markers specific for the organism of interest. Those methods are not without their drawbacks in terms of sample material concentration required or reliable standardization. Digital Droplet Polymerase Chain Reaction (ddPCR) was chosen for our demands as a more elegant, quick and specific method in the investigation of ice nucleation active microorganisms in atmospheric samples. The advantages of ddPCR lie in the simultaneous detection and quantification of genetic markers and their original copy numbers in a sample. This is facilitated by the fractionation of the PCR reaction volumes containing template DNA of ice nucleation active microorganisms from atmospheric samples in thousands of identical droplets. Each droplet encapsulates the reagents necessary for DNA amplification. With template DNA concentrations low enough, the droplets will statistically contain either no template molecules or one molecule. A molecule of template DNA corresponds to exactly one cell of an ice nucleation active microorganism in the original sample provided the genetic marker on the template is present in a single copy. Successful amplification in the presence of template DNA is coupled to a measurable fluorescence signal. The original template DNA concentration is automatically derived from the fraction of fluorescence positive droplets to total droplet number. This far, molecular probes against single-copy genetic markers for ice nucleation active fungi Mortierella alpina, Acremonium implicatum, Isaria farinosa and the ice nucleation active bacterium Pseudomonas syringae have been successfully designed and tested by our group.