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Atmospheric Chemistry and Physics Discussions

Interactive comment on "Using flow cytometry and light-induced fluorescence technique to characterizethe variability and characteristics of bioaerosols in springtime at Metro Atlanta, Georgia" *by* Arnaldo Negron et al.

Anonymous Referee #1

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CONTEXT, ORIGINALITY and GENERAL COMMENTS This manuscript is attempting to answer to a need for the real-time resolution of whole cell microbial bioaerosols, using optical particle recognition (OPR) techniques. The authors present parallel application/assessment of aerosol cytometry (WIBS), conventional liquid cytometry (FCM) and direct microscopy (EPM). The scientific concepts supporting this manuscriptâĂŤresolving the dynamic distributions of airborne microorganisms in "time and space"âĂŤis a reemerging research topic that has important environmental and public health implications for both indoor and outdoor environments. In this reviewer's opinion, this work

has potential to be a significant contribution to the bioaerosol literature at large; however, it needs significant technical revisions prior to publication in any forum, and in its essence is likely not a good fit for the mission of ACP. This disqualification is primarily attributed to the fact that this report is primarily an adaptive technology demonstration and comparison of existing analytical methods. Further, serial (unsupported) assumptions about microbial physiology are embedded in this manuscript, particularly with respect to identifying and quantifying the collected airborne microbiological agents based on presumed genomic characteristics. Important genomic characteristics have either been overly simplified or unfortunately omitted in critical contexts that are needed to support the heart of the work. These (over)simplifications and omissions make it difficult to sustain the author's conclusions given the data they acquired, presented and analyzed (juxtaposition of WIBS, FCM and EPM). The generalization that whole cell bioaerosols can be reliably deconstructed into two pools based on any non-normalized index of DNA/RNA content cannot be not supported by basic microbial (and plant) physiology and the data presented here.

The authors report that a "broad RH range" was monitored during these studies, and that their optical particle recognition data support the conclusion that markedly different microbial populations were airborne under the gross meteorological conditions defined here (T, RH). This may have been the case; however, the data reported are not compelling toward that end. The premise itself is tentative given the somewhat sensational statement that airborne microbes in a "broad" RH range were in-fact monitored, where 40's% < RH < 80's%. A majority of the observations reported (table 1) were under conditions near 50% RH ($\pm$ 9%); this RH is not near saturation conditions, nor is it near desiccating conditions; indeed, many would consider this a "midrange" of relative humidity. In this analytical context, (aut)ecological context or comparative environmental context, by no means is a couple of months of (bio)aerosol sampling conditions in Atlanta "ensuring a wide range of PBAP population(s), state(s) or concentration(s)" (page 6). The retinue of (new) aerobiological acronyms introduced by this manuscript make it difficult to follow at times; many of these (new) terms are not consistent with lexi-

con established over the last generation of bioaerosol research (or molecular biology research for that matter). This includes but is not limited to the following terms: LNA bioLNA HNA First and foremost, all intact (micro)biological cells contain nucleic acids, and the “bio” subscript prefix is conflicted with the fact that environmental nucleic acids can only be of biological origins, regardless of the “quantity” of nucleic acids inside any give (airborne) microbe. In this context, the authors did not acknowledge the fact that DNA is sequestered differently in bacteria, fungi, their spores and pollen grains; that this sequestration is sensitive to RH; and, that the configuration of intracellular DNA has tremendous implications for optical recognition methods and quantitation by FCM, regardless of genetic staining. Further, the authors did not acknowledge the fact that the range of DNA nucleotide pairs (106 – 107 per haploid genome) observed in bacteria, fungi and pollens may not be so different, that they can simply be relegated to “low” and “high” DNA pools by optical staining methods (see Alberts et al, Molecular Biology of the Cell, Section 8.6, Lack of relationship between amount of DNA and organism complexity). In this context, the identity and subsequent quantitation of microbial populations, like the optical DNA reporting methods used here, can be convoluted by the fact that microbial genomes, and common phylogenetic target sequences, are often sequestered intracellularly in markedly different conformations, gross lengths as well as in multiple copies. This is particularly variable across fungal genera where genomes (and their copies) can vary across two orders of magnitude (see Mohanta and Bae, (2015) The diversity of fungal genome. Biol Proced 17:8; and, Lofgren et al (2018), Genome-based estimates of fungal rDNA copy number variation across phylogenetic scales and ecological lifestyles, Molecular Ecology, 10.1111/mec.14995). To support their “low/high” DNA (genome) assignments, and associated microbial classifications, the authors should have, at a minimum, executed some (simple and inexpensive) DNA extractions on at least a subset of their aerosol samples, characterized sentinel sequences (basic qPCR) and juxtaposed this to their optical/cytometry data. In addition to the length/copy number variability presented above, in this midrange of relative humidity , DNA in airborne vegetative cells is in a transitional conformation, while that in

spores is held in a constant conformation regardless of RH (See Peccia et al, 2002, The Effect of Relative Humidity on the UV-induced Induced Inactivation of Airborne Bacteria, Aerosol Science and Technology 35(3):728). For these reasons, this reviewer suggests the work in this manuscript is not appropriate for the ACP readership, because in its essence, it is a comparative demonstration study of existing methods and instrumentation. Further, the manuscript suggests a correlation between fluorescence assignments and microbial phenotype, which are qualitative. For this and other reasons, this reviewer does not believe this is a good fit for the publication mission of ACP. This is does not mean, the work could not be reframed for publication in another journal which is more suited for this type of specialty work I would recommend the following options for the authors to publish this work: (i) condense the work into observation based manuscript (after major revision) as a demonstration study reduced to the constructive criticisms presented. I believe the manuscript has some potential for a good contribution to the bioaerosol literature, but should not be included in or near its present form in ACP (consider resubmission to: Aerosol Science and Technology or Journal of Aerosol Science or Atmospheric Environment).

Hernandez et al. (2016) is cited in text, however it is not presented in the bibliography, Perring et al. (2015) is cited in text, but not presented in bibliography

Specific Technical and Methodological Issues

While Spincon and its newer liquid impingement variants (SpinCon II and OMNI 30000) are recognized as a high volume sampler, these devices, like all impingers, impart a significant pressure drop and associated sampling stresses that are realized differently by bacteria, fungi their spores and pollen grains. While the specific characterization work of SpinConII by Kesavan and coworkers is appreciated, this does not mean the authors can simply dismiss collection stress and sampling efficiency differences, where it cannot be dismissed (Page 7) and the qualification of on (page 22) is convoluted for a reviewer skilled in this art; indeed the SpinCon II correction factors presented on page 22 are at odds with the statement on page 7. At a minimum he authors need

to acknowledge that fungal spores, because of their relative hydrophobicity, have a broad range of impingement retention potential, especially with respect to cells that are not in a sporulated forms (bacteria or fungi), and there are no correction factors that can specifically account for this unless the microbial community structure can be quantitatively apportioned with this equipment (see directly relevant now classic series on variability of bacteria and fungal spore collection/retention efficiency from University of Cincinnati Environmental Health group, circa 1996-2000):

Lin et al, (1999) Collection efficiency and culturability of impingement into a liquid for bioaerosols of fungal spores and yeast cells, Aerosol Science and Technology

Grinshpun et al, (1997) Effect of impaction, bounce and reaerosolization on the collection efficiency of impingers, Aerosol Science and Technology

Lin et al, (1999), Long-term sampling of airborne bacteria and fungi into a non-evaporating liquid, Aerosol Science and Technology

Lin et al, (1998) The effect of sampling time and flow rates on the bioefficiency of three fungal spore sampling methods, Aerosol Science and Technology

While the impingement, flow cytometry methods and DNA intercalating agents used are widely accepted, the their simple extension from aquatic environments (pages 3 and 7)) to generically understanding the "stresses" airborne microbes experience in aerosol environments does not directly support the authors analytical arguments or conclusions. This reviewer found the liquid collection, FCM analyses and microscopy juxtaposition presented by the authors convoluted as it relates to pollen analysis and the generic "two pool" DNA convention presented (i.e. bioLNA and LNA arguments where pollen assignments are concerned). The introduction of Ragweed spp. (page 16), the discussion of its physiology and fragmentation potential, only serves to complicate this matter and since the authors have no independent and definitive observations of pollen community structure from their sample sets, this review recommends condensing the text to omit any specific discussion of individual species. The same

criticism applies to arguments introducing and qualifying pure culture observations of yeasts (Saccharomyces cerevisiae) and bacteria (Pseudomonas aeruginosa) as germane to this investigation. Lastly, this reviewer finds the qualitative descriptions of fluorescence correlations to different microbial phenotypes, and any suggestion that WIBS can "speciate" different airborne microbes, unsupported and inappropriate (page 21). Clearly, the authors are skilled in descriptive statistics and have executed an exhaustive literature on fluorescence-based optical particle recognition instruments. To suggest ABC and HNA are "highly correlated" based on an R2 = 0.4 (figure 4), and that the AB type is "weakly correlated" with HNA where R2 = 0.17, is a subjective presentation of what should be objective thresholds (otherwise these authors need to present interpretive precedents in context and supported their qualitative arguments on why this is "highly" or "weakly" correlated).

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