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August 10, 2015

Via Electronic Mail
No Hardcopy to Follow

FROM: MARK HERNANDEZ, Principal Investigator

RE: RESULTS SUMMARY FOR DYNAMIC PARTITIONING POTENTIAL OF MONODISPURSED PURE CULTURE *Psuedomonas aeruginosa* INTO A PROTOTYPE WATER FEATURE UNDER DEFINED ENVIRONMENTAL CONDITIONS (25C @ 40% RH)

A time series of experiments was performed by aerosolizing known quantities of viable *Psuedomonas aeruginosa* cells into a well characterized bioaerosol chamber (Peccia et al, 2001). The chamber hosted a custom prototype water feature with a reservoir volume of approximately 2L, recycling this reservoir volume across a continuously wetted metal screen of approximately 120 cm^2. Airborne bacterial cells were generated to a concentration > 10^7 m^{-3} over a period of 10 minutes, after which the water feature operation was initiated, and allowed to run in this chamber atmosphere for an hour. The *Psuedomonas aeruginosa* content of the water feature reservoir was quantified prior to and following its operation, facilitating the determination of an overall bioaerosol flux rate from the chamber air into the operating feature.

A *Psuedomonas aeruginosa* strain constitutively expressing green fluorescent protein (GFP) was chosen as the candidate for this study, because pure cultures of this bacterium have been used as a model for the environmental behavior of gram negative bacteria in many military and scientific bioaerosol studies. The GFP variant used here provided for the unambiguous and quantitative tracking of these microbes partitioning from an aerosol phase into an aqueous phase. The tenant defining this approach is as follows: having started these investigations with a clean feature containing sterile water, any GFP-containing microbes present in the reservoir must partition into the water circulating on the screen of the feature. Where executed in a timed series, the flux of microbes into the feature can thus be estimated. Results from challenges with these cells can conservatively approximate partitioning of gram negative bacterial bioaerosols. The growth and contained aerosolization of bacterial cells is a laborious process, and a detailed protocol is referenced in a separate, peer-reviewed document (Peccia, et. al, 2001).

EXPERIMENTAL DESIGN: A stock suspension of pure *Psuedomonas aeruginosa* cells (American Type Culture Collection # ATCC 10145GFP) was raised to log-growth. Immediately following liquid transfer, cells were introduced to a $1m^3$ bioaerosol chamber under slight negative pressure, using ultra-pure nitrogen through a six-jet Collison nebulizer for 10 min, at 24 ^oC, and 40% RH. Following aerosolization, the concentration of these bacteria in the chamber air was estimated by collecting them for two minutes in swirling liquid impingers. This was replicated an hour later. Cells retained in liquid impingers and the water feature's reservoir were counted directly under 1100x magnification using Nikon Eclipse E400 epifluorescence microscope. The quantity of cells was estimated using widely accepted methods (Hernandez et al, 1999 and Henningson et al 1997).

RESULTS: The bioaerosol chamber was loaded with greater than 10 million fresh airborne *Psuedomonas aeruginosa* cells expressing GFP which decayed approximately on order of magnitude in an hour — a level great enough to support observations for partitioning analyses.

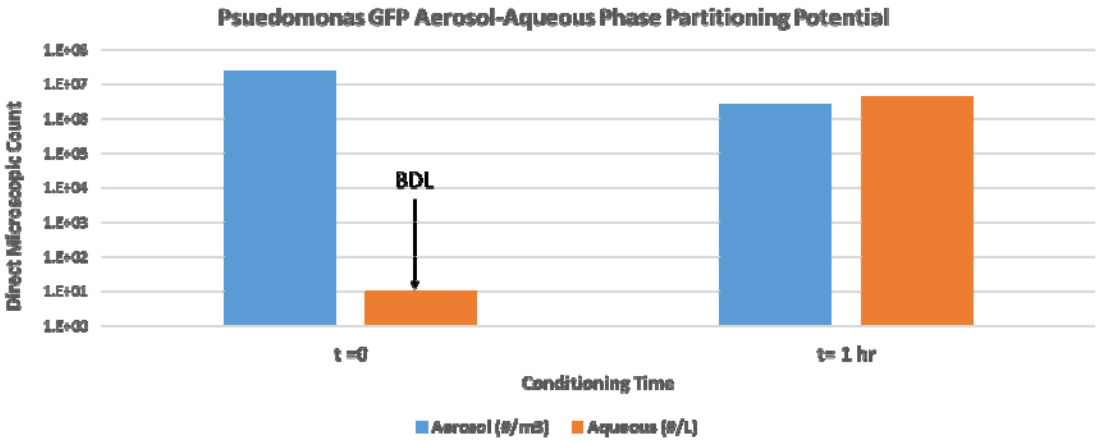

Figure 1. Direct microscopic counts of *Psuedomonas aeruginosa* cells in chamber air (■) at 24 ºC and 40% RH, and in the fiberglass water feature reservoir (■). Bar height represents averaged count per media volume (m^3 for air, or L for water).

DISCUSSION: Exposure to bioaerosol under these chamber conditions caused a significant increase the concentration of *P. aeruginosa* cells in the fiberglass reservoir of the prototype water feature. As judged by direct microscopic count, bioaerosol chamber exposure increased the total cell numbers in the water feature by c.a. 10^6/L when challenged with an aerosol initially containing 2.5 x 10^7 *P. aeruginosa* cells over the period of an hour. When controlled for aging effects, aerosol hosting otherwise healthy *Psuedomonas aeruginosa* cells appeared to partition into the operating water feature at a gross rate of c.a. 10^4 cm^{-2} hr^{-1}.

Henningson, E. W., M. Lundquist, E. Larsson, G. Sandstrom, and M. Forsman, 1997, A comparative study of different methods to determine the total number and the survival ratio of bacteria in aerobiological samples.: Journal Aerosol Science, v. 28, p. 459

Hernandez, M., S. L. Miller, D. W. Landfear, and J. M. Macher, 1999, A Conmbined Fluorochrome Method for Quantitation of Metabolically Active and Inactive Airborne Bacteria: Aerosol Science and Technology, v. 30, p. 145.

Peccia, J., Werth, H., Miller, S. L., and Hernandez., M. 2001 The Effect of Relative Humidity on the UV-induced Induced Inactivation of Airborne Bacteria. *Aerosol Science and Technology* v. 35 p. 728-740.