LETTER

Inadequate methods and questionable conclusions in atmospheric life study

Our aerobiology team was pleased to read "Microbiome of the upper troposphere: Species composition and prevalence, effects of tropical storms, and atmospheric implications" by DeLeon-Rodriguez et al. (1). On the opposite side of the continent, at a different time of year, and using other molecular methods, we also found rich bacterial assemblages in the upper troposphere capable of long-range transport (2, 3). We were delighted to see the convergence and hope it will inspire follow-up investigations because the field truly needs a global monitoring network to enhance understanding of patterns and implications. However, we have some concerns about the work by DeLeon-Rodriguez et al. (1).

First, the *Materials and Methods* (both in the article and *SI Materials and Methods*) of DeLeon-Rodriguez et al. (1) were inadequate for follow-up studies. Publishing air-pump specifications is absolutely critical for standardizing aerobiology methods. No details or citations of the air pump used onboard the DC-8 aircraft were provided. In addition, the authors do not describe how the system prevents contamination. How was the wall of the aircraft penetrated with the collection tubes? How did they handle the pressure differential from inside the DC-8 and the external ambient air pressure? What about a vendor name, part number, or images for the pump and filtration systems? How was sterility within the intake-plumbing lines and filters maintained from take-off until sampling altitudes?

Second, most previous tropospheric studies have concluded that microbes are diluted in the atmosphere. Our recent work used filters and a high-volume air pump just like DeLeon-Rodriguez et al. (1). We processed on average 360 m^3 of ambient air per sample and measured about 6–20 cells (2). DeLeon-Rodriguez et al. (1), in comparison, only pumped on average 6 m^3 of ambient air per sample, yet the authors measured about 5,100 cells. It seems strange that a pump 60-times less efficient than ours would capture ∼1,000 times more cells. Perhaps the air-filtered column values listed in Table S1 of DeLeon-Rodriguez et al. (1) were miscalculated? There seem to be other errors in the same table. For example, how could bacterial contribution be 276% on September 16, 2010?

Third, referring to the possibility of the atmosphere as an ecosystem, the authors concluded "...it is conceivable that these groups could remain metabolically active in clouds" (1). The line between viability and active growth/metabolism was blurred with this conclusion. There is no evidence in this report that microorganisms can metabolize at the extreme low temperatures in the upper troposphere. We cannot understand why temperatures at sampling altitudes were not reported in the article. At equivalent heights in the upper troposphere the temperature could be in the range of −50 °C to −70 °C. No known microbe on Earth can grow or metabolize at such a low temperature, even if it could theoretically use organic compounds in the sky. Active metabolism, growth, or replication may be plausible in clouds at lower altitudes, but observing these activities was not achievable with the methods used by DeLeon-Rodriguez et al. (1).

Finally, DeLeon-Rodriguez et al. concluded that "...bacteria are at least two orders of magnitude more abundant relative to fungi at high altitudes" (1). However, the timing of the sampling flights (August and September) was never discussed. Lower fungal numbers would be expected in the late summer, and a seasonal influence should have been addressed before arriving at such a conclusion. Most Northern Hemisphere fungi release reproductive spores in the springtime, and indeed our work during that season in 2011 measured a more even ratio of airborne bacteria to fungi (2).

We were disappointed that these basic issues were overlooked during peer review.

***David Joseph Smith*[a,1] *and Dale Warren Griffin*[b]**

[a]*National Aeronautics and Space Administration Surface Systems Office, Kennedy Space Center, FL 32899; and* [b]*US Geological Survey, Coastal and Marine Science Center, St. Petersburg, FL 33701*

1 DeLeon-Rodriguez N, et al. (2013) Microbiome of the upper troposphere: Species composition and prevalence, effects of tropical storms, and atmospheric implications. *Proc Natl Acad Sci USA* 110(7):2575–2580.
2 Smith DJ, et al. (2012) Free tropospheric transport of microorganisms from Asia to North America. *Microb Ecol* 64(4):973–985.
3 Smith DJ, et al. (2013) Intercontinental dispersal of bacteria and archaea by transpacific winds. *Appl Environ Microbiol* 79(4): 1134–1139.

Author contributions: D.J.S. and D.W.G. wrote the paper.

The authors declare no conflict of interest.

[1]To whom correspondence should be addressed. E-mail: david.j.smith-3@nasa.gov.