Is the lung microbiome alive? Lessons from Antarctic soil

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Is the lung microbiome alive? What we can learn from 1) the cold, dry, salty soil of Antarctica and 2) an elegant new translational study in the *European Respiratory Journal*. A new essay by @robertpdickson and @jenn_bak_. https://bit.ly/3p76a0L

Cite this article as: Baker JM, Dickson RP. Is the lung microbiome alive? Lessons from Antarctic soil. *Eur Respir J* 2021; 58: 2100321 [DOI: 10.1183/13993003.00321-2021].

Received: 2 Feb 2021
Accepted: 3 Feb 2021

> *"…we have seen no living thing, not even a moss or a lichen… It is certainly a valley of the dead; even the great glacier which once pushed through it has withered away."*
> Captain Robert Falcon Scott, on the absence of life in Taylor Valley, Antarctica, 1903 [1]

> *"The normal lung is free from bacteria."*
> *Robbins Pathologic Basis of Disease*, 6th Edition, 1999 [2]

Before studying an ecosystem, it's first worth asking whether it exists. For much of the 20th century, this was the challenge faced by microbiologists interested in Antarctic soil bacteria. Restricted both by the challenges of sampling and by limited cultivation techniques, researchers believed that Antarctic soil was devoid of living microbes. The low numbers of detected bacterial cells were attributed to "contamination" from wind-borne species, which seeded the soil but displayed little evidence of viability [3]. Microbial life in the cold, dry, salty soil was so sparse that NASA used the polar deserts of the McMurdo Dry Valleys as testing sites for the life-seeking technology deployed in the Viking missions to Mars (figure 1) [4, 5].

We now know that Antarctic soil contains viable and active microbial life. These communities, though fewer in number and simpler in structure than their counterparts in temperate soils, are surprisingly diverse, metabolically active (with few exceptions [6, 7]), and well-adapted to their particular, though inhospitable, niches (table 1) [8]. This evolution in our understanding of Antarctic soil microbiota was propelled by the application of molecular microbiology techniques (*e.g.* 16S rRNA gene amplicon sequencing and metagenomics), which identify microbial taxa undetected by conventional cultivation [9]. Complementary studies using biochemical methods and advanced cultivation have confirmed the viability of these microbes, but their specific metabolic activity, and their ecological influence on their local microenvironment, remains an active area of study [10].

If this recent history of Antarctic soil microbiology sounds familiar, it is because it tightly parallels the recent revolution in our understanding of the lung microbiome. For most of the 20th century, limited by our dependence on culture-based techniques, we assumed that healthy lungs are too inhospitable an environment for bacteria. We deemed the lungs sterile, and ascribed any detected microbes to contamination (*i.e.* "oral flora") [11]. In the past decade, however, the application of sequencing-based techniques to respiratory specimens has revealed that even healthy lungs (in humans and animals alike) harbour diverse and dynamic low-biomass bacterial communities [12, 13]. Like Antarctic soil, lungs are not as barren and microbially lifeless as we thought.

Yet DNA sequencing-based techniques, for all their advantages, cannot answer a key and pressing question: are these bacterial communities alive? DNA is a stable and persistent molecule, and its detection

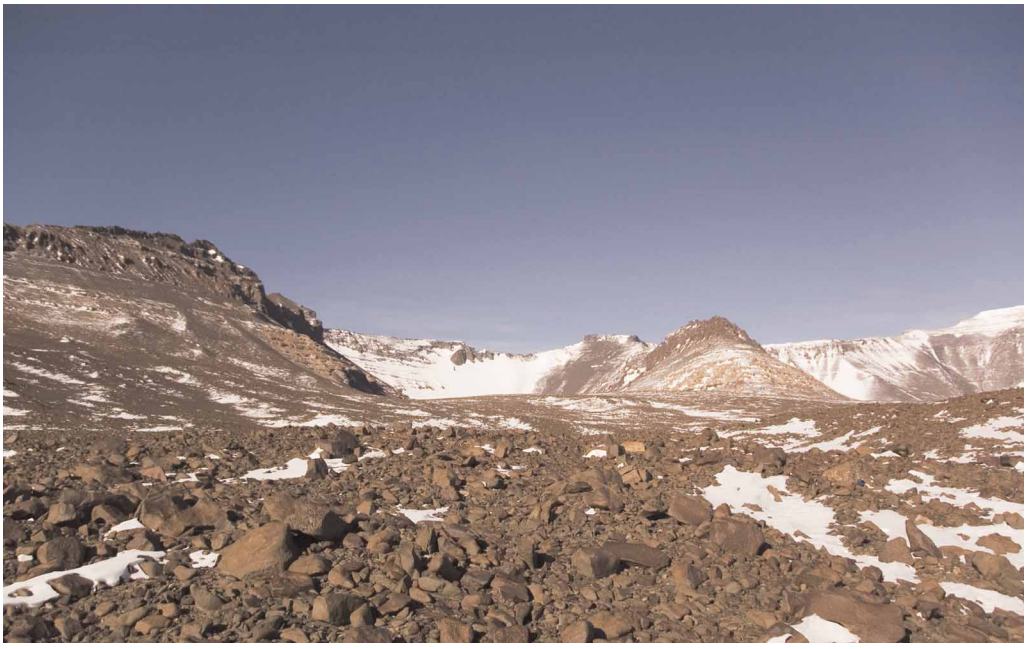

FIGURE 1 The inhospitable soils of the Antarctic desert in Beacon Valley, the highest and driest location in the McMurdo Dry Valleys. Photograph courtesy of James Dickson.

does not discriminate between living and dead microbial cells. The lung microbiome field has approached this question indirectly by establishing the lung microbiome's biological and clinical significance: variation in lung bacterial communities has been shown to correlate with pulmonary immune tone [13–16], disease development and clinical outcomes [17–19], and exposure to antibiotics and environmental conditions [13, 20, 21]. However suggestive, none of these discoveries directly establishes the viability and metabolic activity of lung bacteria in health. The question remains: is the lung microbiome alive?

In this issue of the *European Respiratory Journal*, Sulaiman *et al.* [22] address this question by directly interrogating the transcriptional and metabolic activity of bacteria detected in healthy mammalian lungs. The authors studied a cohort of healthy human smokers and exhaustively characterised bacterial communities and metabolic profiles in bronchoalveolar lavage (BAL) fluid. The authors employed three complementary methods to simultaneously characterise the identity and metabolic capacity of bacterial communities: 1) *inferred* metabolic potential based on taxonomic assignments from 16S gene amplicon sequencing (DNA-based); 2) *confirmed* metabolic potential using whole genome sequencing (DNA-based); and 3) transcriptomically *active* metabolic pathways using RNA-seq (RNA-based). The authors then compared these *inferred*, *confirmed* and *active* microbial metabolic profiles to the directly measured quantity of short-chain fatty acids (SCFAs) detected in the same specimens. This parallel approach allowed them to determine which sequencing method correlates most strongly with *actual* microbial metabolic products. Additionally, in an illuminating experiment, they challenged the lungs of healthy mice with a mixture of human oral bacteria and compared the relative rates of clearance of microbial DNA and RNA. Taken together, this approach equipped the investigators to determine both the viability and temporal stability of bacterial communities in healthy lungs.

The authors made three fundamental observations, each of considerable methodological and conceptual importance. The first and most foundational finding was that lower respiratory tract microbiota are indeed transcriptionally and metabolically active in human lungs, even in the absence of pulmonary infection. While variable across subjects, the overall correlation between the *identity* of lung microbiota and their *transcriptional* and *metabolic* products was convincing, and clearly reflects active metabolism. Secondly, distinct sequencing-based methods yielded variable correlations with concentrations of alveolar metabolites, reflecting differential accuracy in identifying viable, metabolically active bacteria. In the specific case of SCFAs, direct characterisation of microbial transcription (*via* RNA-seq) corresponded more closely with actual alveolar metabolite concentrations than did DNA-based techniques. Thirdly, in their experimental model of oropharyngeal aspiration, DNA from aspirated bacteria was far more persistent

TABLE 1 Ecological comparison of microbial communities in terrestrial soil and the mammalian lung

	Habitat: terrestrial soil		Habitat: mammalian lungs	
	Antarctic desert	**Tropical and temperate**	**Healthy**	**Diseased**
Microbial biomass	Low	High	Low	High
Habitability of microenvironment	Inhospitable • low nutrient availability • cold, dry, salty	Hospitable • high nutrient availability • warm, moist	Inhospitable • low nutrient availability • antimicrobial peptides lining airway surfaces • intact cellular immunity	Hospitable • high nutrient availability • impaired fluid and microbial clearance • dysfunctional cellular immunity
Microbe–microbe interactions	Sparse, indirect • *via* biogeochemical effects on the environment and limited direct interactions	Common, complex • *via* direct and indirect competition and cooperation	Sparse, indirect • *via* indirect effects on the host response	Common, complex • *via* direct and indirect competition and cooperation (*e.g.* quorum sensing, priming of host defence)
Stability of microbial community composition	Stable, low turnover • limited by low replication rates and low immigration	Stable, low turnover • limited by niche-adapted "climax communities"	Dynamic, rapid turnover • highly dynamic, subject to continuous immigration and elimination	Stable, low turnover • persistent and stable, dominated by niche-adapted "colonizers"
Microbial activity	Present • metabolically active, though variable due to dormancy and local conditions	Present • metabolically active	Present • metabolically active, though variable due to dynamic immigration and elimination	Present • metabolically active
Microbial effects on local microenvironment	Significant • nutrient cycling (*e.g.* atmospheric nitrogen fixation, autotrophic carbon fixation)		Unknown • calibration of innate and adaptive immune defences [13, 15, 27] • modulation of epithelial cell function? • others?	Significant • nutrient metabolism and metabolite production • induction and perpetuation of proinflammatory response • epithelial cell injury

(days) than their transcribed RNA (hours), illustrating the dynamic and transient nature of the viable lung microbiome in health.

The study's core finding has profound implications for our evolving understanding of the microbial ecology of the lungs. While the transcriptional activity of respiratory bacteria has been explicitly studied in the context of infection [23, 24], this study reveals that lung bacteria exert a metabolic influence on their microenvironment even in the absence of disease. These results are congruent with the prior observation that when appropriately varied culture conditions are applied to lung specimens from healthy subjects, the majority of taxa identified by sequencing are viable by culture [25]. Further, the differential resolution between DNA- and RNA-based characterisation (considered with the murine time course experiment) provides clarity to our understanding of the temporal dynamics of lung ecology. Unlike bacterial communities in the lower gut or diseased airways, the lung microbiome in health is not "resident," but is instead defined by a dynamic "steady state" determined by the balance of frequent immigration (from oropharyngeal aspiration) and constant elimination pressure (from cough, mucociliary clearance and host defences [26]). Healthy lungs may not be sterile, but they are trying hard to be!

This study also further confirms the immunological significance of lung microbiota in health. The microbiome-derived metabolites detected in BAL fluid (*e.g.* acetate, propionate, isovalerate) have pleiotropic effects on lung immunity, including modulating effects on alveolar macrophages and neutrophils [27–29]. Both in healthy humans and animals, variation in lung microbiota correlates with variation in baseline lung immune tone [13, 15]. The current study's investigators recently demonstrated that the same oral commensal bacteria used in the study's murine aspiration model also induce a persisting MyD88-dependent Th17 response that is protective against subsequent pneumococcal pneumonia (an effect not observed when instilling dead bacterial cells) [30]. However transient viable lung microbiota may be, their immunological effects are persistent and significant. While the gut microbiome has received considerable attention for its production of immunomodulatory metabolites (and its role in the purported "gut–lung axis"), the current study makes clear that we can no longer ignore the local metabolic contributions of lung microbiota on maintaining immune homeostasis.

Given the inherent differences between the compared sequencing-based approaches, their variable accuracy in predicting actual microbial metabolism is unsurprising. Metagenomic- and metatranscriptomic-derived

community data correlated much more strongly with measured metabolites than did those inferred from 16S rRNA gene amplicon sequencing. This finding is intuitive, as the amplicon-based approach relies on inferred metabolic potential based only on coarse taxonomic classification (*i.e.* not on directly sequenced metabolism-related genes). These results provide a convincing and useful corrective to over-interpretation of 16S-derived data that professes to characterise microbial metabolism. However, the current study should itself not be over-interpreted as implying that there is no longer a role for amplicon sequencing in studying the lung microbiome. As summarised above, amplicon-based sequencing has consistently and reproducibly confirmed the biological, immunological and prognostic significance of the lung microbiome in health and disease. As an accessible, affordable and high-throughput means of characterising bacterial communities, amplicon sequencing still belongs in our toolkit. By analogy, immunologists have not abandoned flow cytometry despite the advent of single cell sequencing. Despite this caution, the current study does convincingly show that for *functional* study of the lung microbiome, amplicon-based approaches are insufficient.

The current study is not without limitations. While adequate for a proof-of-concept study, the limited number of subjects and the heterogeneity of signal make it difficult to discern how uniform and prevalent these microbial and metabolic signatures are among healthy subjects. The significance of "background-predominant" taxa in many of the human cohort samples is unclear, and may be an indirect marker of undetectably low microbial signal (due perhaps to variation across subjects, differences in the frequency of subclinical aspiration, or the investigators' use of acellular BAL fluid [31]). Future studies with larger cohorts and serial sampling will hopefully address many remaining unknowns, including the temporal stability of the lung communities within healthy individuals.

Caveats aside, this important study should definitively silence any remaining skepticism regarding the viability and metabolic significance of the lung microbiome in health. Despite their harsh environments, both Antarctic soil bacteria and the healthy lung microbiome manage to persist and function at the edge of the habitable. In both cases, the natural next question – after establishing their existence – is how these microbial communities in turn affect their local microenvironments. Now that we have confirmed microbial existence in the extreme environment of the healthy lung, we are poised to determine how this ecosystem keeps us healthy and contributes to disease. Our expedition into the supposed "valley of the dead" was well worth the trip.

Acknowledgements: The authors thank Jay Dickson for sharing both the image of Beacon Valley as well as his infectious passion for Antarctic research. J.M. Baker would especially like to thank her former research advisor, Matt Sattley, for the chance to study bacteria from the McMurdo Dry Valleys during her undergraduate years, sparking her enduring interest in microbiology.

Conflict of interest: J.M. Baker has nothing to disclose. R.P. Dickson has nothing to disclose.

References

1 Scott RF. The Voyage of the "Discovery". London, Smith, Elder, & Co., 1905.
2 Cotran R, Kumar V, Collins T, *et al.* Robbins Pathologic Basis of Disease. 6th Edn. Philadelphia, Saunders, 1999.
3 Horowitz NH, Cameron RE, Hubbard JS. Microbiology of the dry valleys of Antarctica. *Science* 1972; 176: 242–245.
4 Horowitz NH, Hubbard JS, Hobby GL. The carbon-assimilation experiment: the Viking Mars lander. *Icarus* 1972; 16: 147–152.
5 Levin GV, Ann Straat P. Labeled release – an experiment in radiorespirometry. *Origins Life Evol Biosphere* 1976; 7: 293–311.
6 Goordial J, Davila A, Lacelle D, *et al.* Nearing the cold-arid limits of microbial life in permafrost of an upper dry valley, Antarctica. *ISME J* 2016; 10: 1613–1624.
7 Dragone NB, Diaz MA, Hogg I, *et al.* Exploring the boundaries of microbial habitability in soil. *bioRxiv* 2020; preprint [https://doi.org/10.1101/2020.08.03.234583].
8 Cowan DA, Makhalanyane TP, Dennis PG, *et al.* Microbial ecology and biogeochemistry of continental Antarctic soils. *Front Microbiol* 2014; 5: 154.
9 Smith JJ, Tow LA, Stafford W, *et al.* Bacterial diversity in three different Antarctic cold desert mineral soils. *Microb Ecol* 2006; 51: 413–421. .
10 Cary SC, McDonald IR, Barrett JE, *et al.* On the rocks: the microbiology of Antarctic dry valley soils. *Nat Rev Microbiol* 2010; 8: 129–138.

11 Dickson RP, Erb-Downward JR, Martinez FJ, *et al.* The microbiome and the respiratory tract. *Ann Rev Physiol* 2016; 78: 481–504.
12 Hilty M, Burke C, Pedro H, *et al.* Disordered microbial communities in asthmatic airways. *PLoS ONE* 2010; 5: e8578.
13 Dickson RP, Erb-Downward JR, Falkowski NR, *et al.* The lung microbiota of healthy mice are highly variable, cluster by environment, and reflect variation in baseline lung innate immunity. *Am J Respir Crit Care Med* 2018; 198: 497–508.
14 Segal LN, Alekseyenko AV, Clemente JC, *et al.* Enrichment of lung microbiome with supraglottic taxa is associated with increased pulmonary inflammation. *Microbiome* 2013; 1: 19.
15 Segal LN, Clemente JC, Tsay J-CJ, *et al.* Enrichment of the lung microbiome with oral taxa is associated with lung inflammation of a Th17 phenotype. *Nat Microbiol* 2016; 1: 16031.
16 O'Dwyer DN, Ashley SL, Gurczynski SJ, *et al.* Lung microbiota contribute to pulmonary inflammation and disease progression in pulmonary fibrosis. *Am J Respir Crit Care Med* 2019; 199: 1127–1138.
17 Invernizzi R, Barnett J, Rawal B, *et al.* Bacterial burden in the lower airways predicts disease progression in idiopathic pulmonary fibrosis and is independent of radiological disease extent. *Eur Respir J* 2020; 55: 1901519.
18 Dickson RP, Schultz MJ, van der Poll T, *et al.* Lung microbiota predict clinical outcomes in critically ill patients. *Am J Respir Crit Care Med* 2020; 201: 555–563.
19 Combs MP, Wheeler DS, Luth JE, *et al.* Lung microbiota predict chronic rejection in healthy lung transplant recipients: a prospective cohort study. *Lancet Respir Med* 2021; 9: 601–612.
20 Fillion-Bertrand G, Dickson RP, Boivin R, *et al.* Lung microbiome is influenced by the environment and asthmatic status in an equine model of asthma. *Am J Respir Cell Mol Biol* 2019; 60: 189–197.
21 Ashley SL, Sjoding MW, Popova AP, *et al.* Lung and gut microbiota are altered by hyperoxia and contribute to oxygen-induced lung injury in mice. *Sci Transl Med* 2020; 12: eaau9959.
22 Sulaiman I, Wu BG, Li Y, *et al.* Functional lower airways genomic profiling of the microbiome to capture active microbial metabolism. *Eur Respir J* 2021; 58: 2003434.
23 Chaffin DO, Taylor D, Skerrett SJ, *et al.* Changes in the *Staphylococcus aureus* transcriptome during early adaptation to the lung. *PLoS ONE* 2012; 7: e41329.
24 Damron FH, Oglesby-Sherrouse AG, Wilks A, *et al.* Dual-seq transcriptomics reveals the battle for iron during *Pseudomonas aeruginosa* acute murine pneumonia. *Sci Rep* 2016; 6: 39172.
25 Venkataraman A, Bassis CM, Beck JM, *et al.* Application of a neutral community model to assess structuring of the human lung microbiome. *mBio* 2015; 6: e02284-14.
26 Dickson RP, Erb-Downward JR, Huffnagle GB. Towards an ecology of the lung: new conceptual models of pulmonary microbiology and pneumonia pathogenesis. *Lancet Respir Med* 2014; 2: 238–246.
27 Segal LN, Clemente JC, Wu BG, *et al.* Randomised, double-blind, placebo-controlled trial with azithromycin selects for anti-inflammatory microbial metabolites in the emphysematous lung. *Thorax* 2017; 72: 13–22.
28 Eftimiadi C, Tonetti M, Cavallero A, *et al.* Short-chain fatty acids produced by anaerobic bacteria inhibit phagocytosis by human lung phagocytes. *J Infect Dis* 1990; 161: 138–142.
29 Corrêa-Oliveira R, Fachi JL, Vieira A, *et al.* Regulation of immune cell function by short-chain fatty acids. *Clin Transl Immunol* 2016; 5: e73.
30 Wu BG, Sulaiman I, Tsay J-CJ, *et al.* Episodic aspiration with oral commensals induces a MyD88-dependent, pulmonary Th17 response that mitigates susceptibility to *Streptococcus pneumoniae*. *Am J Respir Crit Care Med* 2021; 203: 1099–1111.
31 Dickson RP, Erb-Downward JR, Prescott HC, *et al.* Cell-associated bacteria in the human lung microbiome. *Microbiome* 2014; 2: 28.