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BRIEF REPORT

Culturable bacteria in clouds at Réunion, tropical island

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Abstract The viable bacterial assemblages in clouds at Réunion Island (Indian Ocean) were examined through culture-based approach. A total of 176 isolates were recovered from 15 independent cloud events collected during 3 field campaigns, and identified to the species level through full length 16S rRNA gene sequencing. As often in atmospheric samples, Alpha-, Beta- and Gamma-proteobacteria dominated, along with Actinobacteria, Firmicutes, and Bacteroidetes, depicting these as the backbone of the global aeromicrobiome. A comparative analysis with similar work from a cloud sampling site in Central France revealed site-specificities, and numerous common species. These latter included members of *Pseudomonas*, *Sphingomonas*, *Bacillus*, *Staphylococcus*, *Rhodococcus* and others, whose such widespread presence in clouds supports the existence of a pan-atmospheric microbiome. This also confirms that cultures remain powerful methods in the description of the viable microbial diversity by allowing deep taxonomic affiliation.

Keywords Bacteria · Clouds · Tropical Island

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1 Introduction

Clouds carry diverse viable bacteria and fungi, with epidemiological, ecological, and biogeophysical implications (Šantl-Temkiv et al., 2022). Over the last decade in aerobiology, as in other environmental sciences, molecular methods have been extensively used to investigate microbial assemblages at the expense of culture-based approaches. Nevertheless, these latter offer valuable information concerning the viable fraction of the community. They provide unique chances to explore survivors of the atmospheric microbiome under laboratory conditions, for their physiology, biological traits, and metabolic capacities (e.g., Joly et al., 2015). Cultured specimens also represent new prospects for biotechnological applications

(Sarmiento-Vizcaíno et al., 2018), and they provide reference genomes that can support omic studies (Besaury et al., 2017). Rare specimens, not detected by molecular methods, can also eventually be grown and characterized as required for describing new taxa.

Given the numerous trophic modes that exist in bacteria, cultures are necessarily selective for a fraction of the viable community. It is thus of first importance that similar culture media are used when comparing distinct studies. As cultivability from complex environmental microbial assemblages is improved by oligotrophic conditions (Vartoukian et al., 2010), investigations of atmospheric samples often involve oligotrophic media such as R2A (Fahlgren et al., 2010; Vaïtilingom et al., 2012), which was originally designed for prospecting bacteria in potable water. Our research group isolated culturable bacteria from clouds collected at puy de Dôme summit in Central France for nearly 2 decades using such medium. Here, we aim at expanding this documentation in a drastically different environmental context, the aeromicrobiome of which remains largely unexplored: along Mt Maïdo slopes at Reunion, a small tropical island situated in the Indian Ocean at ~ 700 km East from Madagascar. Similar protocols as those at our usual sampling site were used, so as to allow direct comparison of the data.

2 Material and methods

Cloud water samples were collected along the slopes of Mont Maïdo, Réunion Island, during 3 field campaigns led in the springs 2014, 2015 and 2019. Two different locations situated 3 km apart from each other were investigated depending on the precise location of clouds (Piste Omega (21°03′26″ S, 55°22′05″ E, 1,760 m a.s.l., and Mt Maïdo observatory, 21°4′39″ S, 55°22′59″ E, 2,160 m a.s.l.). Autoclaved aluminum cloud droplet impactors with an approximate cut-off diameter of 7 µm were deployed from masts at heights of up to 10 m above ground, in order to reach cloud layers. Sampling was performed for 20–220 min, during which 50–150 mL of cloud water were recovered under sterile conditions and processed for analyses. A complete description of the sampling operations and images of the cloud sampling apparatus can be found in (Dominutti et al., 2022).

Triplicate volumes of 0.1 mL of each cloud water sample were inoculated on R2A medium (DIFCO) and incubated at ambient room temperature (19–25 °C) in the dark. Visually distinct colonies were further isolated after 4–7 days of incubation, by successive cultures on the same medium at 17 °C. Bacteria isolates were first discriminated from eukaryotes (yeasts) by optical microscope observation, and identified based on their 16S rRNA gene sequences. Genomic DNA was extracted from isolated colonies resuspended into 200 µL sterile distilled H_2O, either by mechanical cell disruption (3 cycles of 100 °C for 1 min then − 80 °C for 1 min, with 20 s vortexing between cycles), or, for recalcitrant strains, using DNeasy Power Water Kit (Qiagen) following manufacturer's recommendations from 100 µL of cell suspension. PCR amplification of 16S rRNA genes was realized in reaction mixes composed of (final concentrations): 1.5 mM $MgCl_2$ (final concentration), 5 µL of 10X buffer (Qiagen HotStarTaq buffer), 0.2 mM of each dNTP (Sigma), 0.4 µM of forward and reverse primers, 2.5 U of *Taq* DNA Polymerase (Fisher ref. FB600065, or "Hot-Start" Qiagen reference 203,203) and 1–2 µL of DNA extract, into a total volume of 50 µL. The universal bacteria ribosomal gene primers 27F (5′-AGAGTTTGATCC TGGCTCAG-3′) and 1492r (5′-GGTTACCTTGTT ACGACTT-3′) were used (Fredriksson et al., 2013). PCR amplification reactions were as follows: initial denaturation at 95 °C for 3 min (or 15 min for the "Hot Start" Taq Pol.), followed by 30 cycles of 45 s at 94 °C, 45 s at 53 °C and 45 s at 72 °C, and a final elongation step of 7 min at 72 °C (10 min for the "Hot Start" Taq Pol.). The PCR products (~ 1450 bp) were verified for length by electrophoresis on 0.8% agarose gel, then purified using GenElute PCR Clean-Up kit (Sigma) or QIAquick PCR Purification kit (Qiagen). DNA was finally quantified by absorbance at 260 nm using BiospecNano (Shimadzu) and normalized to 5 ng μL^{-1} for Sanger sequencing (Eurofins MWG) using the same primers as for PCR reactions. The forward and reverse sequences obtained for each isolate were reassembled using BioEdit 7.0.5.3 (Hall, 1999). Consensus sequences were aligned, clusterized and annotated with Mothur v1.46.1 (Schloss et al., 2009) against SILVA v132 database (Quast et al., 2013). The corresponding sequence files have been deposited in GenBank and have the accession numbers MW711672-MW711715, MW711381-MW711451

and MW631945-MW632049. Only full length 16S rRNA gene sequences were considered in this analysis.

3 Results and discussion

3.1 Diversity of heterotrophic culturable bacteria in clouds at Réunion Island

Fifteen independent cloud water samples were examined for culturable bacteria on R2A medium. From these, a total of 176 visually distinct colonies were isolated (2 to 33 per sample), corresponding to 139 amplicon sequence variants (ASV, full rRNA gene sequence) distributed into 84 $OTU_{0.01}$ (~species level). As often in atmospheric samples, most isolates were pigmented in yellow, brown–beige, orange or pink. Pigments confers protection against UV-light and oxidants; and thus, they potentially favor microbial survival in the atmosphere (Tong & Lighthart, 1997).

About half of the isolates (87/176) were Proteobacteria (62% Gamma-Proteobacteria, 30% Alpha-Proteobacteria and 8% Beta-Proteobacteria), followed by Actinobacteria (36%) and Firmicutes (11%). The most frequent genera were *Bacillus*, *Sphingomonas*, *Pseudomonas* and *Curtobacterium*, representatives of which were cultured from > 40% of the samples. *Sphingomonas* and *Pseudomonas* exhibited high intraspecific richness, with 8 and 10 distinct $OTU_{0.01}$ identified from 15 and 17 isolates, respectively (< 2 isolates/$OTU_{0.01}$). In turn, *Bacillus* and *Curtobacterium* exhibited low richness, with only 5 and 2 $OTU_{0.01}$ from 14 isolates each (2.8 and 7 isolates/$OTU_{0.01}$, respectively). *Stenotrophomonas* and *Microbacterium* also contributed numerous isolates (21 and 16, respectively); whereas, these were less frequently detected (30% of the samples) and they exhibited low or moderate intraspecific diversity (7 and 3.2 isolates/$OTU_{0.01}$). Other frequent and/or diverse taxa included *Rhodococcus*, *Streptomyces*, *Arthrobacter*, *Glutamicibacter*, *Chryseobacterium*, and *Acinetobacter*. On the other hand, 25 genera were represented by one single $OTU_{0.01}$ retrieved from 1, or exceptionally 2 samples.

Most of the taxa represented here are ubiquitous bacteria. Their presence in atmospheric samples is commonly reported, and related with inputs from soils, rhizosphere, or phyllosphere. Many of these are of particular interest for their versatility, possibly linked with increased survival during atmospheric transport, and for their possible interactions with atmospheric chemical and physical processes (biotransformation, ice nucleation, etc.) (e.g., Delort et al., 2010).

3.2 Comparison with mid-latitude clouds at the continental site of puy de Dôme Mountain

For several years, our research group has investigated culturable microbes from clouds collected at the mid-latitude continental rural site of puy de Dôme Mountain summit, in Central France (1465 m a.s.l.) (Amato et al., 2007; Vaïtilingom et al., 2012). Figure 1 compares the phylum level distributions of bacteria isolates from clouds collected at both sites. The two profiles are very similar, with only slightly more Firmicutes at Réunion, at the expense of Alphaproteobacteria. The difference might relate with the temperature of incubation, 19–25 °C for Réunion, *versus* 17 °C at puy de Dôme.

Despite these apparent resemblances, specifying the distribution of $OTU_{0.01}$ (~bacteria species) between the 2 sites depicts clear differences (Fig. 2). At maximum 24% of the $OTU_{0.01}$ in each phylum were shared between the sites. Most Gamma-Proteobacteria, Firmicutes, Actinobacteria and Beta-Proteobacteria were specific of Réunion Island; whereas, most Alpha-Proteobacteria and Bacteroidetes were specific of puy de Dôme. Shared $OTU_{0.01}$ were affiliated with *Pseudomonas* and *Stenotrophomonas* in Gamma-Proteobacteria, *Bacillus* and *Staphylococcus* in Firmicutes, *Sphingomonas* and *Methylobacterium* in Alpha-Proteobacteria, *Pedobacter* in Bacteroidetes, and *Curtobacterium*, *Rhodococcus*, *Streptomyces*, *Plantibacter*, *Microbacterium* and *Salinibacterium* in Actinobacteria. This supports the particular propensity of species in these taxa to be part of the global atmospheric microbiota.

At both sites, *Sphingomonas* (Alpha-Proteobacteria) and *Pseudomonas* (Gamma-Proteobacteria) dominated in diversity (number of OTUs) and in biomass (number of isolates), and *Bacillus* (Firmicutes), *Rhodococcus*, and *Streptomyces* (Actinobacteria) were among the most represented taxa (Fig. 3). In turn, some genera, not necessarily the rarest, appeared site-specific, such as *Acinetobacter*

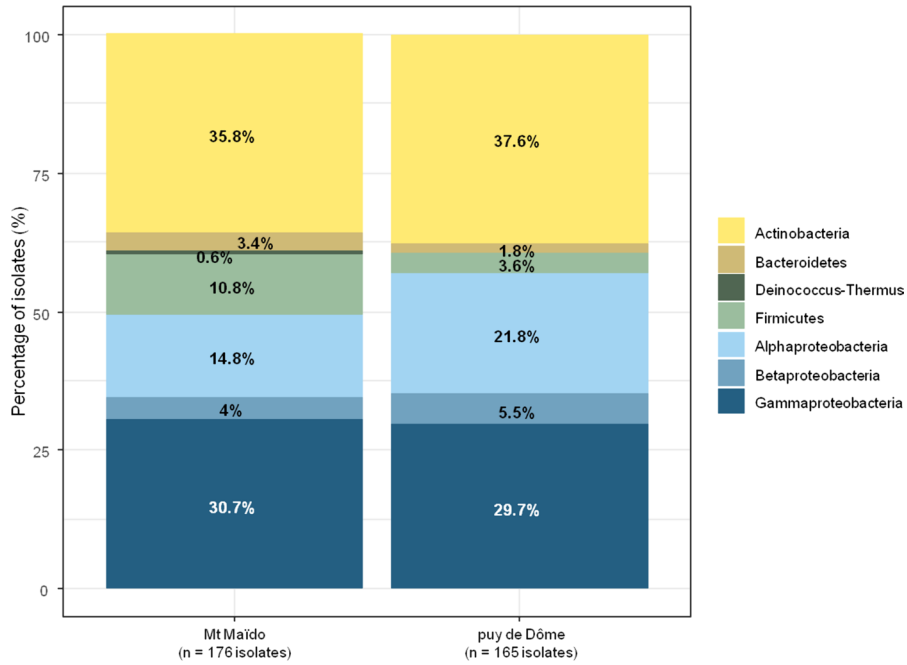

Fig. 1 Phylum level distribution of bacteria isolates in clouds at Réunion (this study) and at puy de Dôme Mountain (data from Vaïtilingom et al., 2012)

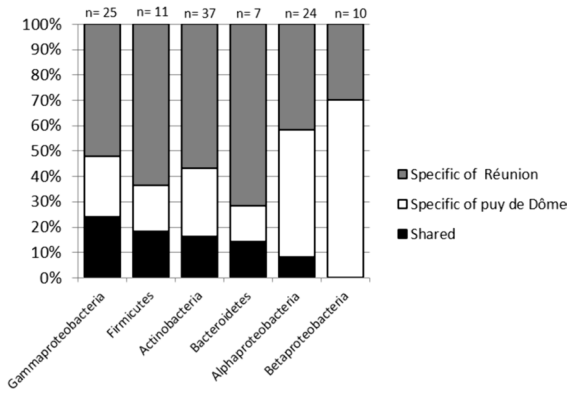

Fig. 2 Distribution of OTU diversity between clouds at Réunion and at puy de Dôme. Only isolates with full length 16S rRNA gene sequences were considered here

at Réunion, and *Clavibacter* and *Xanthomonas* at puy de Dôme. Although concluding on the total absence of certain microbial groups is hazardous here, the structure of the viable culturable assemblages clearly differed between the two sites, and this was only visible at deep taxonomic levels reachable through isolates.

4 Conclusions

Although these may not be representative of the whole community structure, culture methods allow deep taxonomic affiliations, and these are thus methods of choice for disentangling microbial assemblage specificities. These generally remain hidden using high-throughput sequencing methods for assessing microbial diversity given the low taxonomic resolution offered. The presence of similar viable species of bacteria in clouds at very distant places and different contexts supports these as core members of a possible pan-atmospheric microbiome.

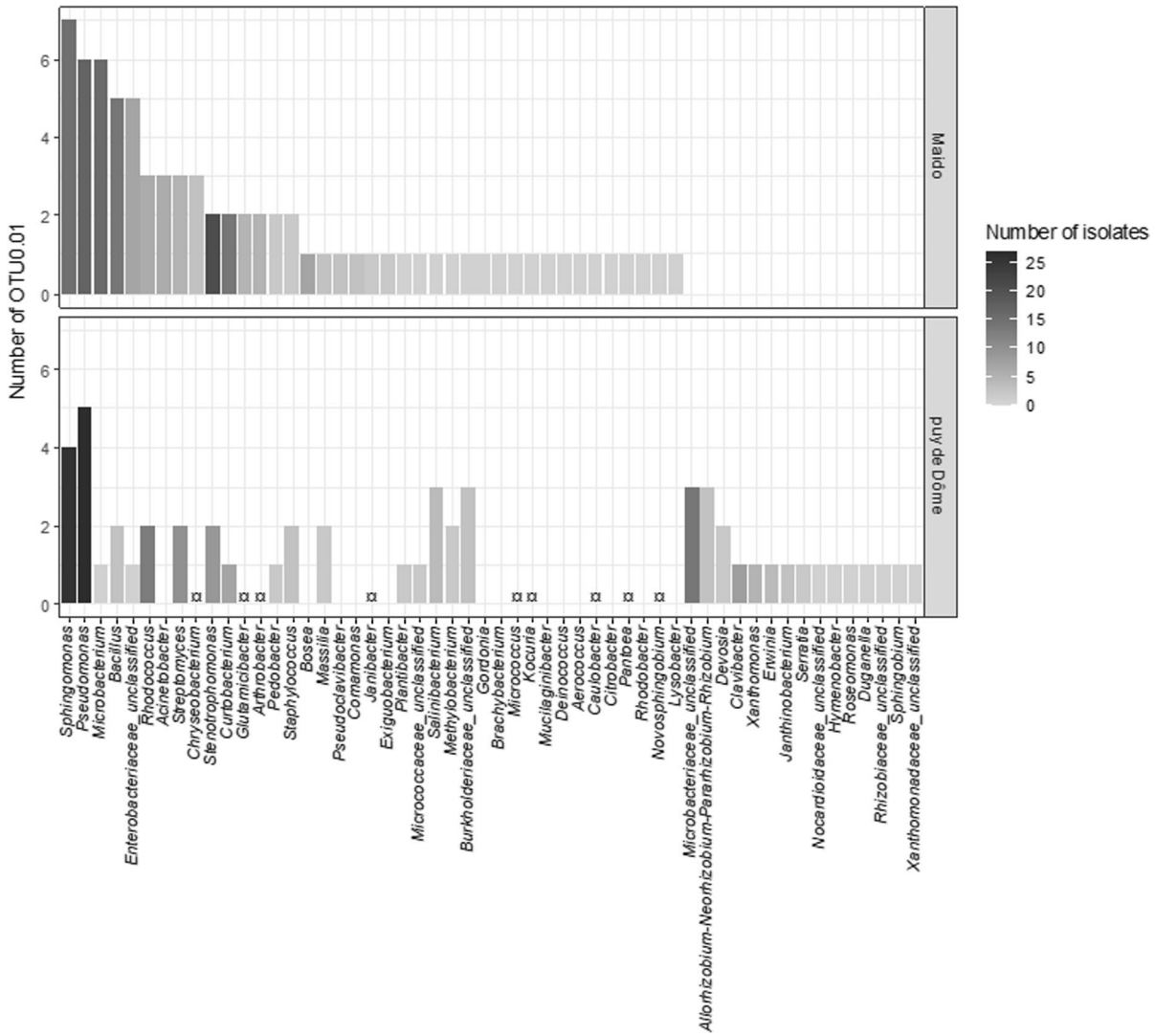

Fig. 3 Taxonomic affiliation of bacteria isolates at the genus level, ordered by decreasing number of OTUs per genus, in clouds at Réunion (upper panel, this study), and corresponding data for clouds at puy de Dôme (lower panel, data from (Vaïtilingom et al., 2012) and unpublished work). An asterisk indicates that representatives of the corresponding genus were recovered by culture but not sequenced for full length rRNA gene sequence so these could not be assessed by taxonomic clustering

Author contribution ML, LD and AMD designed the study; MJ, MV, MB and LD performed cloud sampling; TC, MJ, CJ, MS, MA, MV, CG, MB performed strain isolations and identifications; TC, MJ, and PA performed data analysis; TC, MJ and PA wrote the manuscript.

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Data availability The sequence files used in this study have been deposited in GenBank (https://www.ncbi.nlm.nih.gov/nuccore) and have the accession numbers MW711672-MW711715, MW711381-MW711451 and MW631945-MW632049.

Declarations

Conflict of interest The authors have no financial or proprietary interests in any material discussed in this article**.**

References

Amato, P., Parazols, M., Sancelme, M., Laj, P., Mailhot, G., & Delort, A.-M. (2007). Microorganisms isolated from the water phase of tropospheric clouds at the Puy de Dôme: Major groups and growth abilities at low temperatures. *FEMS Microbiology Ecology, 59*(2), 242–254. https://doi.org/10.1111/j.1574-6941.2006.00199.x

Besaury, L., Amato, P., Sancelme, M., & Delort, A. M. (2017). Draft genome sequence of pseudomonas syringae PDD-32b-74, a model strain for ice-nucleation studies in the atmosphere. *Genome Announcements, 5*(30), e00742-e817. https://doi.org/10.1128/genomeA.00742-17

Delort, A.-M., Vaïtilingom, M., Amato, P., Sancelme, M., Parazols, M., Mailhot, G., et al. (2010). A short overview of the microbial population in clouds: Potential roles in atmospheric chemistry and nucleation processes. *Atmospheric Research, 98*(2–4), 249–260. https://doi.org/10.1016/j.atmosres.2010.07.004

Dominutti, P. A., Renard, P., Vaïtilingom, M., Bianco, A., Baray, J.-L., Borbon, A., et al. (2022). Insights into tropical cloud chemistry in Réunion (Indian Ocean): Results from the BIO-MAÏDO campaign. *Atmospheric Chemistry and Physics, 22*(1), 505–533. https://doi.org/10.5194/acp-22-505-2022

Fahlgren, C., Hagström, Å., Nilsson, D., & Zweifel, U. L. (2010). Annual variations in the diversity, viability, and origin of airborne bacteria. *Applied and Environmental Microbiology, 76*(9), 3015–3025. https://doi.org/10.1128/AEM.02092-09

Fredriksson, N. J., Hermansson, M., & Wilén, B.-M. (2013). The choice of PCR primers has great impact on assessments of bacterial community diversity and dynamics in a wastewater treatment plant. *PLoS ONE*. https://doi.org/10.1371/journal.pone.0076431

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignement editor and analysis program for Windows 95/98/NT, vol. 41(2), pp. 95–98.

Joly, M., Amato, P., Sancelme, M., Vinatier, V., Abrantes, M., Deguillaume, L., & Delort, A.-M. (2015). Survival of microbial isolates from clouds toward simulated atmospheric stress factors. *Atmospheric Environment, 117*, 92–98. https://doi.org/10.1016/j.atmosenv.2015.07.009

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research, 41*(D1), D590–D596. https://doi.org/10.1093/nar/gks1219

Šantl-Temkiv, T., Amato, P., Casamayor, E. O., Lee, P. K. H., & Pointing, S. B. (2022). Microbial ecology of the atmosphere. *FEMS Microbiology Reviews*. https://doi.org/10.1093/femsre/fuac009

Sarmiento-Vizcaíno, A., Espadas, J., Martín, J., Braña, A. F., Reyes, F., García, L. A., & Blanco, G. (2018). Atmospheric precipitations, hailstone and rainwater, as a novel source of streptomyces producing bioactive natural products. *Frontiers in Microbiology*. https://doi.org/10.3389/fmicb.2018.00773

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., et al. (2009). Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology, 75*(23), 7537–7541. https://doi.org/10.1128/AEM.01541-09

Tong, Y., & Lighthart, B. (1997). Solar radiation is shown to select for pigmented bacteria in the ambient outdoor atmosphere. *Photochemistry and Photobiology, 65*(1), 103–106.

Vaïtilingom, M., Attard, E., Gaiani, N., Sancelme, M., Deguillaume, L., Flossmann, A. I., et al. (2012). Long-term features of cloud microbiology at the puy de Dôme (France). *Atmospheric Environment, 56*, 88–100. https://doi.org/10.1016/j.atmosenv.2012.03.072

Vartoukian, S. R., Palmer, R. M., & Wade, W. G. (2010). Strategies for culture of 'unculturable' bacteria: Culturing the unculturable. *FEMS Microbiology Letters*. https://doi.org/10.1111/j.1574-6968.2010.02000.x