Microb Ecol (2016) 72:305–312
DOI 10.1007/s00248-016-0767-z

ENVIRONMENTAL MICROBIOLOGY

Presence of Archaea in the Indoor Environment and Their Relationships with Housing Characteristics

Sepideh Pakpour[1,2] · **James A. Scott**[3] · **Stuart E. Turvey**[4,5] · **Jeffrey R. Brook**[3] · **Timothy K. Takaro**[6] · **Malcolm R. Sears**[7] · **John Kloronomos**[1]

Received: 3 April 2016 / Accepted: 5 April 2016 / Published online: 20 April 2016

Abstract Archaea are widespread and abundant in soils, oceans, or human and animal gastrointestinal (GI) tracts. However, very little is known about the presence of Archaea in indoor environments and factors that can regulate their abundances. Using a quantitative PCR approach, and targeting the archaeal and bacterial 16S rRNA genes in floor dust samples, we found that Archaea are a common part of the indoor microbiota, 5.01 ± 0.14 (log 16S rRNA gene copies/g dust, mean ± SE) in bedrooms and 5.58 ± 0.13 in common rooms, such as living rooms. Their abundance, however, was lower than bacteria: 9.20 ± 0.32 and 9.17 ± 0.32 in bedrooms and common rooms, respectively. In addition, by measuring a broad array of environmental factors, we obtained preliminary insights into how the abundance of total archaeal 16S rRNA gene copies in indoor environment would be associated with building characteristics and occupants' activities. Based on the results, Archaea are not equally distributed within houses, and the areas with greater input of outdoor microbiome and higher traffic and material heterogeneity tend to have a higher abundance of Archaea. Nevertheless, more research is needed to better understand causes and consequences of this microbial group in indoor environments.

Keywords Archaea · Bacteria · Indoor environment · qPCR · Building characteristics · Human activities

✉ Sepideh Pakpour
spakpour@mit.edu

1 Department of Biology, University of British Columbia, Kelowna, BC, Canada

2 Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA

3 Dalla Lana School of Public Health, University of Toronto, Toronto, ON, Canada

4 Department of Pediatrics, University of British Columbia, Vancouver, BC, Canada

5 Child & Family Research Institute, BC Children's Hospital, Vancouver, BC, Canada

6 Faculty of Health Sciences, Simon Fraser University, Vancouver, BC, Canada

7 Faculty of Health Sciences, McMaster University, Hamilton, ON, Canada

Introduction

The biology and ecology of the third domain of life, Archaea, have been studied far less when compared to the other domains including bacteria and eukarya. Archaea are microorganisms discovered in the late 1970s [1]. For years after their discovery, scientists believed that archaea were restricted to extreme environments, such as deep-sea hydrothermal vents, hypersaline waters, or strictly anoxic ecosystems [2]. Development of culture-independent molecular techniques and high-throughput molecular sequencing approaches transformed this belief by illustrating their presence, often with high abundance and diversity, in terrestrial and aquatic environments [3–5], animal care facilities [6–8], deteriorated medieval wall paintings [9], as well as the human and animal microbiome such as gastrointestinal (GI) tracts [10–14] and human oral cavities [15]. However, the presence of archaea in many other ecosystems has still been investigated scarcely and our understanding of their role in their habitat is limited.

One such overlooked ecosystem is the indoor built environment. There is significant ongoing interest in better understanding the "built environment microbiome" [16], with a focus on characterizing microbial diversity as well as the environmental parameters that would drive its patterns [16–26].

Nevertheless, most of the past studies on the indoor microbiome considered mainly bacteria [16, 17, 27–30] and, to a lesser degree, fungi [19, 20, 23, 25, 31, 32]. Here, we used culture-independent molecular approaches to study the archaea in indoor dust from homes in the so-called "miniCHILD" study, which is a preliminary cohort of 54 homes in the Vancouver area recruited to assist in the optimization and validation of data collection tools for the larger Canadian Healthy Infant Longitudinal Development (CHILD) study [33, 34]. We sought to answer three general questions: (1) Are archaea regular components of built environment microbiomes? If yes, (2) what would be their magnitude compared to indoor bacteria? And (3) how would building characteristics and occupants' activities relate to the variation of archaeal abundances?

Material and Methods

Sample Collection

Between May 2008 and May 2009, trained research assistants collected dust from the homes of families with newborn children using a sterile, depyrogenated custom-designed aluminum collection device attached to the end of a vacuum cleaner (Model S3680, Sanitaire Canister Vac, Charlotte, NC, USA). The collection device held two nylon DUSTREAM filters (Indoor Biotechnologies Inc, Charlottesville, VA). Two dust samples were collected in each house; the first sample was a composite of the mattress and floor in the room where the subject child slept, and the second sample was collected from the floor of the room occupied most often by the family. A standardized floor area was initially sampled (2 m^2), and if insufficient dust was obtained, the sampling area was expanded. Research technicians visually observed the thimbles after vacuuming 2 m^2; if the thimbles were less than half-full, the technician continued vacuuming in a new area of the room until the required amount was met. The exact size of the vacuumed area was recorded for all samples taken. Samples were then fractionated using a sterile depyrogenated 100 Mesh sieve (~150 μm), and the fine fraction transferred to a sterile depyrogenated borosilicate glass vial with a Teflon-lined screw cap (VWR 1 dram glass vial, West Chester, PA) and stored at −80 °C until analysis.

DNA Extraction and Quantitative PCR Analyses

Total DNA was extracted from 100 mg of collected fine dust samples using a FastDNA® SPIN Kit for Soil (MP Biomedicals, LLC, Solon, OH, USA), which was selected systematically by using the Order Preference by Similarity to Ideal Solution (TOPSIS) method [35] as the most optimum extraction kit for dust samples in the present case study. Subsequently, extracted DNA samples were checked for integrity by agarose gel electrophoresis with Lambda DNA HindIII Digest standards (New England BioLabs, Ipswich, MA, USA), and their quantities were measured using the QuantiFluor® dsDNA System (Promega, Madison, WI, USA). The purity of extracted DNA samples was evaluated by measuring each sample's ratio of the optical density at 260 and 280 nm using the NanoVue Plus™ spectrophotometer (GE Healthcare, Buckinghamshire, UK), before preserving them at −20 °C. Abundances of both archaeal and bacterial 16S rRNA gene copy numbers were measured by quantitative PCR (qPCR); using A364aF (5′ CGGGGYGCASCAGGCGCGAA 3′) and A934bR (5′ GTGCTCCCCCGCCAATTCCT 3′) primers for archaea [36] and BACT1369F (5′ CGGTGAATACGTTCYCGG 3′) and PROK1492R (5′ GGWTACCTTGTTACGACTT 3′) for bacteria [37]. Although the abundance of 16S gene sequences is not a surrogate measure of the relative abundance of the archaeal and bacterial cells containing those sequences (because of variations in genomic copy number of the 16S gene in microbial species), in the rest of this manuscript for the sake of brevity, 16S rRNA gene copy numbers will be referred to as archaeal/bacterial abundances.

All PCR amplifications were carried out in a CFX96 Touch™ Real-Time PCR Detection System (BioRad, Ontario, Canada) and each PCR reaction mixture (20 μL) contained 10 μL of SsoFast™ EvaGreen® Supermix (Biorad, Hercules, CA), 1.5 μL of 1000 μg/μL T4 gene 32 protein (Biolabs, Ipswich, MA), 0.4 μM of each primer, nuclease-free water (IDT, Coralville, IA, USA), and 2 μL of extracted DNA (5 ng/μL). Thermal-cycling conditions for 16S archaea were as follows: 95 °C for 2 min for the enzyme activation, 40 cycles of 95 °C for 30 s (denaturation), and 61.5 °C for 30 s (annealing and extension), followed by 1 cycle of melting analysis (65–95 °C (0.1 °C/2 s)). These conditions for 16S bacteria included: 95 °C for 2 min for the enzyme activation, 40 cycles of 95 °C for 30 s (denaturation), and 56 °C for 30 s (annealing and extension), followed by 1 cycle of melting analysis (65–95 °C (0.1 °C/2 s)).

Standard curves were obtained using three replicates of 1:10 serial dilutions of linearized plasmids containing both cloned archaeal and bacterial 16S rRNA sequences, giving a concentration range from 10 to 10^6 copies/μL. Amplification efficiencies of 92.2–94.7 % ($R^2 > 0.985$) and 90.1–105.8 ($R^2 > 0.963$) were observed for archaeal and bacterial standards, respectively. Finally, melting curve analyses at the end of all qPCR runs and agarose gel running of qPCR products were performed to check for amplification and specificity of the products.

Collection of Environmental Variables and Statistical Analyses

We monitored and recorded 668 housing characteristics as well as building inhabitant activities by using standardized

questionnaires and direct on-site visits for the purpose of statistical analyses. An exhaustive list of these factors has been described in recent publications [33, 34], and a subset is shown in Table 1. The questionnaire was comprised of questions on the location, history, and characteristics of the unit, such as basic house dimensions, construction details of the building envelope, furniture materials, and finishes for interior designs; the occurrence of factors which could influence moisture sources and air change as well as number, type, and activities of the occupants.

Statistical analyses were performed in PRIMER 7 and STATICTICA 12 [38, 39]. Regarding the first two questions (listed in the Introduction), the abundance of archaeal and bacterial genes in bedrooms versus the most used rooms were first plotted (in log scale) to illuminate the indoor archaeal abundance relative to that of bacteria. Subsequently, a Wilcoxon matched pairs test was used to investigate whether or not there is a significant statistical difference between total archaeal abundances in different types of rooms. Then, for the third question of the study, the BEST (Bio-Env) routine, namely BVSTEP, was used to determine which of 668 environmental factors and resident activities "collectively" best explain the overall variation in archaeal total abundances in both room types. Subsequently, the significance of the BEST analysis

Table 1 Sub-sample of 668 collected environmental factors

Building design characteristics	Type and density of occupants	Occupants' activities
Age of ceiling	Number of adults in the house	Frequency of bathroom fan usage
Age of floor	Number of children in the house	Frequency of house cleaning
Age of house	Number of plants	Frequency of keeping the child bedroom's window open
Basement condition	Number of visitors/day	Frequency of keeping the most used room's window open
Basement dampness	Presence of long-hair cats	Hanging clothes inside the house
Basement foundation	Presence of long-hair dogs	Presence of stuff toys
Child room area (sq. m)	Presence of short-hair cats	Type of vacuum
Child room carpet area (sq. m)	Presence of short-hair dogs	Usage level of gas fireplace
Child room wall cover	Presence of plants	Usage level of radiators
Child room window cover	Presence of moth in house	Use of chemical spray and cloth
Cleanliness of basement	Presence of mouse in house	Use of garden sprays/weed killers
Condensation on bedroom Windows in cooler weather	Presence of pets	Use of mop
Evidence of leak in the house	Furniture and equipment	Use of unscented or scented candles
Finished basement or added insulation	Number of pieces of leather	Use of antibacterial hand cleaner
Floor level	Number of pieces of metal	Use of broom
Furnace age	Number of pieces of solid woods	Use of chemical sprays for cleaning
Furnace condition	Number of plastic/vinyl furniture	
Most used room area (sq. m)	Number of press wood furniture	Use of disinfectants
Most used room carpet area (sq. m)	Presence of air conditioning system	Use of feather duster
Number of rooms in the house	Presence of electronic devices	Use of floor cleaners
Presence of garage	Presence of humidifier	Use of glass cleaners
Type of garage	Presence of stove fan in kitchen	Use of liquid or solid air fresheners
Presence of swimming pool	Outdoor related	Use of multi-surface cleaners
Presence of upgraded plumbing system	Geographic distance	Use of oven cleaners
Total volume of the house	Is the house within 100 m of: Body of water	Use of plug-in deodorizers
Type of flooring	Factory	Use of plumbing cleaners
Type of foundation	Farm	Use of scented laundry detergents
Type of fuel in the house	Gas station	Use of spray air fresheners
Type of furnace's filter	Major highway/artery	Use of toilet bowl cleaners
Type of garage	Other source of pollution	Use of vacuum
Type of house	Neighbor currently doing renovations	Use of wet cloth (water only) for cleaning
Type of insulation		Use of swiffer wet jet
Type of lawn		
Type of wall covering		

result was validated through a permutational null distribution to ensure that the selected combinations of environmental variables were not obtained by chance. Univariate data analyses, namely Mann-Whitney (for two-level categorical factors), Kruskal-Wallis (for multi-level categorical variables), and Spearman Correlation tests (for numerical variables) were next employed to explore which individual screened environmental variable would be relatively more associated with the variation of archaeal abundances.

Results

Archaeal abundances varied between 5.01 ± 0.14 (log 16S rRNA gene copies/g dust, mean ± SE) in bedrooms and 5.58 ± 0.13 in the most used rooms. However, these magnitudes were notably lower than indoor bacteria, which were between 9.20 ± 0.32 in bedrooms and 9.17 ± 0.32 in the most used rooms (Fig. 1). When we compared sample pairs (bedroom and the most used room of the same houses), a significant difference was detected between their archaeal abundances (Wilcoxon matched pairs test, $p = 0.04$), with higher abundance occurring in the most used rooms (Fig. 1a). However, no similar indication was found for the indoor bacteria (Fig. 1b). Subsequently, by using the BEST procedure, we found that almost 55 % of variation of total magnitudes of indoor archaea can be explained by 15 and 21 out of 668 environmental factors in bedrooms and the most used rooms, respectively (Table 2). When the relative effect size of screened factors by BEST for bedrooms was estimated individually, however, only "use of electric dryer, vented outdoors" (Mann-Whitney U test, $p = 0.005$) remained significant and negatively associated with the total abundances of bedrooms' archaea (Fig. 2a). Association of this factor was also noted in the most used rooms, albeit to a lesser degree ($p = 0.06$, Table 2 and Fig. 2b). Moreover, most used rooms' archaeal abundances were significantly associated with the presence of upgraded plumbing systems ($p = 0.029$), hanging wet clothes inside the house ($p = 0.031$), and the use of liquid or solid air fresheners ($p = 0.032$). In particular, it was found that the presence of an upgraded plumbing system (Fig. 2c) and hanging wet clothes inside the house (Fig. 2d) was negatively correlated with the total abundance of archaea in the most used rooms. In contrast, the use of liquid or solid air fresheners was positively associated with the total abundance of archaea in the most used rooms (Fig. 2e).

Discussion

We have demonstrated the presence of archaea in the house dust and the influence of selected indoor characteristics on archaeal abundance. These data may add to the existing knowledge that archaea are not only present in extreme environments with physical limits for biological systems [1, 2], but they are also broadly distributed and abundant in moderate environments [3, 7, 15, 40–44]. The latter can include Methanomicrobiales and Thermoplasmatales in freshwater and marine habitats [45], Crenarchaeota and Thaumarchaeota in soil [45, 46], and methanogens in the human and animal intestinal tracts [10–14].

Earlier studies have shown that archaea comprise a significant proportion of microbes in soil and pelagic ocean waters, with a ratio of archaea/bacteria around 1:10 [46, 47]. In floor dust, we observed a much smaller archaeal contribution with a ratio of archaea/bacteria around 0.02:10 in bedrooms and 0.06:10 in the most used rooms. One of the explanations might be that we used fine dust particles for sampling, while it

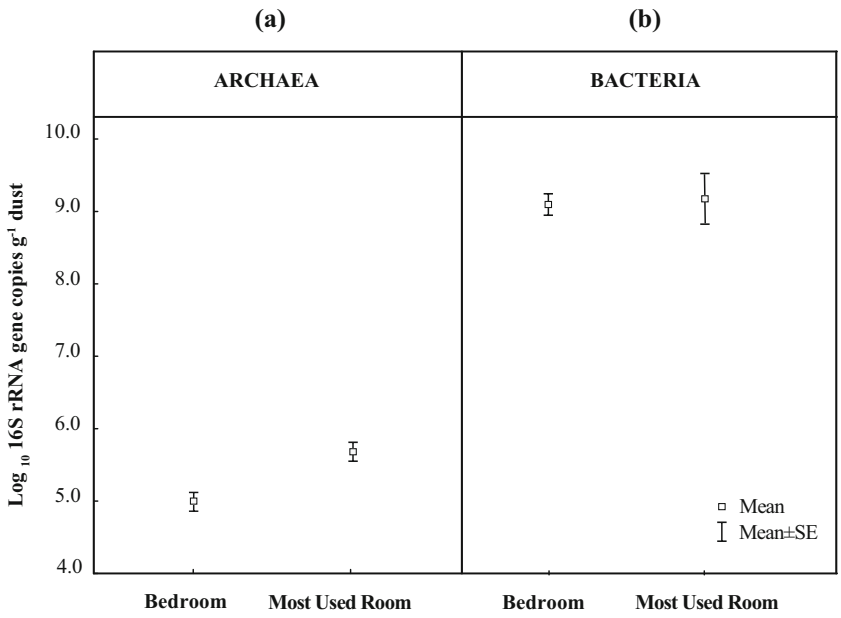

Fig. 1 Total abundance of **(a)** archaea and **(b)** bacteria in bedrooms and the most used rooms

Table 2 Environmental and behavioral factors that best explain variation of the total archaeal abundance in bedrooms and the most used rooms

Screened factors by BEST[a]	
Bedroom	The most used room
Numeric	Numeric
Room area (sq. m)	Number of plastic or vinyl furniture
Number of plastic or vinyl furniture	
Number of press wood furniture	
Number of leather furniture	
Categorical	Categorical
Do they take off shoes when enters the unit	Age of floor
Is there a private child room	Basement condition
Occurrence of condensation on windows in cooler weather	Basement foundation
Presence of humidifier	Hanging wet clothes inside the house
Presence of long hair cat	Presence of air conditioning system
Presence of stuff toys	Presence of garage
Type of furnace's filter	Presence of plants in home
Type of window's covering	Presence of plastic or vinyl covered furniture
Use of Electric dryer, vented outdoors	Presence of short hair cat
Use of gas fire place	Presence of stove fan in the kitchen
Use of swiffer wet jet	Presence of swimming pool
	Presence of upgraded plumbing system
	Use of antibacterial hand cleaner
	Use of broom
	Use of floor cleaners
	Use of electric dryer, vented outdoors
	Use of liquid or solid air fresheners
	Use of oven cleaners
	Use of scented laundry detergents
	Use of vacuum

[a] Multi-factor analyses: All factors are collectively responsible for 55.1 % ($p = 0.03$) and 56.3 % ($p = 0.02$) variation of total abundance of archaea in bedrooms and the most used rooms, respectively

has been suggested that archaeal traces are mostly present in coarse particles [48]. The fact that archaea were less numerous in indoor dust may also indicate that the indoor archaeal assemblages are mostly allochthonous (passive entrants of archaeal traces such as Halobacteriales, Thermoplasmatales, and the members of Thaumarchaeota

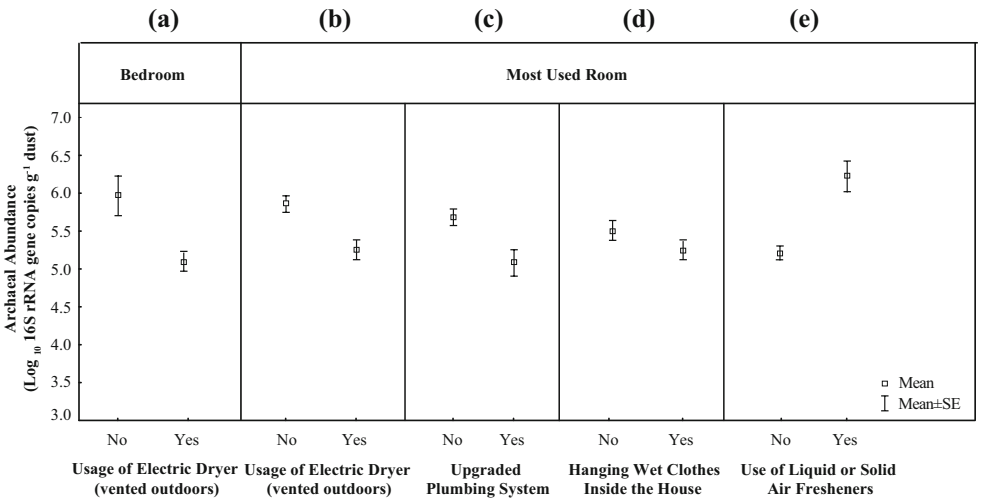

Fig. 2 **(a)** The relationship between uses of electric dryer vented outdoors and total archaeal abundance in bedrooms. The relationships between **(b)** uses of electric dryer, vented outdoors, **(c)** presence of upgraded plumbing system, **(d)** hanging wet clothes inside house, and **(e)** use of liquid or solid air fresheners and total archaeal abundance in most used rooms

[48] brought inside along with the fresh air through windows and ventilation systems or on the shoes and clothing of inhabitants). This is in contrast to the indoor bacterial assemblages, which are a mixture of both allochthonous and autochthonous assemblages (live and active inhabitants of dust). In addition, we found that archaea, within houses, are not equally distributed and the most used rooms had significantly higher total archaeal abundances than bedrooms (Fig. 1a). This may be because of the higher human traffic and a greater input of outdoor archaea propagated indoors through open windows, on footwear, or other items brought inside.

Within each room type, the total abundance of archaea varied, depending on different environmental factors. For example, the use of an electric clothes dryer, vented outdoors was negatively correlated with the total abundance of archaea (Fig. 2a, b). One of the explanations might be that every time a laundry load is dried, some archaea may be removed from the indoor environment through exhaust fans, and hence, the neighboring areas in the house would contain lower amount of these microorganisms. In addition, we found that in houses where wet clothes were hung inside (Fig. 2d), the total abundance of archaea was lower. This could be because when clothes are hung indoors to dry (as opposed to outdoors), the indoor environment may have lower input of outdoor air and thus a lower input of airborne archaea.

Finally, in addition to outdoor sources, some specific indoor sources may contribute to the abundance of indoor archaea. For example, the use of liquid or solid air fresheners inside houses was positively associated with the total abundance of archaeal sequences (Fig. 2e). One explanation may be that archaeal traces are embedded in the raw materials and additives of air fresheners and, hence, distributed into the indoor environment upon freshener usage. Also, houses with old plumbing systems showed higher levels of archaea (Fig. 2c), likely because of the accumulation of archaeal biofilm [49, 50] inside the plumbing system where biofilm-forming species can survive, release, and disperse into the indoor environment.

In summary, this study provides evidence that archaea are present in household dust, and their abundances may be associated with the physical building characteristics, occupant activities, and product use. The results may be further used to form the basis of intervention studies assessing the causality between factors and total abundance of indoor Archaea, diversity of the indoor archaeal community by using throughput-sequencing methods, as well as studies focusing on determining association of the indoor archaeal community with human health and disease. Better understanding of indoor microbial diversity can eventually provide more awareness into the role of environment as a determinant of health, particularly in relation to non-infectious diseases in which inflammatory mediators are believed to be important.

Acknowledgments The authors are very grateful to all the families who participated in this study, and the whole miniCHILD study team, which included interviewers, laboratory technicians, research scientists, and volunteers. Useful discussions and valuable insight of Dr. Louise Nelson and Ms. Geet Hans from the University of British Columbia are greatly acknowledged. We also acknowledge the financial assistance from the Natural Sciences and Engineering Research Council of Canada (Discovery Grants) and the CHILD Study.

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

References

1. Woese CR, Kandler O, Wheelis ML (1990) Towards a natural system of organisms—proposal for the domains archaea, bacteria, and eucarya. Proc Natl Acad Sci U S A 87:4576–4579. doi:10.1073/pnas.87.12.4576
2. Schleper C, Jurgens G, Jonuscheit M (2005) Genomic studies of uncultivated archaea. Nat Rev Microbiol 3:479–488. doi:10.1038/nrmicro1159
3. Delong EF, Wu KY, Prezelin BB, Jovine RVM (1994) High abundance of archaea in antarctic marine picoplankton. Nature 371:695–697. doi:10.1038/371695a0
4. Timonen S, Bomberg M (2009) Archaea in dry soil environments. Phytochem Rev 8:505–518. doi:10.1007/s11101-009-9137-5
5. Bengtson P, Sterngren AE, Rousk J (2012) Archaeal abundance across a pH gradient in an arable soil and its relationship to bacterial and fungal growth rates. Appl Environ Microbiol 78:5906–5911. doi:10.1128/aem.01476-12
6. Lecours PB, Veillette M, Marsolais D, Duchaine C (2012) Characterization of bioaerosols from dairy barns: reconstructing the puzzle of occupational respiratory diseases by using molecular approaches. Appl Environ Microbiol 78:3242–3248. doi:10.1128/aem.07661-11
7. Just N, Lecours PB, Marcoux-Voiselle M, Kirychuk S, Veillette M, Singh B, Duchaine C (2013) Archaeal characterization of bioaerosols from cage-housed and floor-housed poultry operations. Can J Microbiol 59:46–50. doi:10.1139/cjm-2012-0305
8. Nehme B, Gilbert Y, Letourneau V, Forster RJ, Veillette M, Villemur R, Duchaine C (2009) Culture-independent characterization of archaeal biodiversity in swine confinement building bioaerosols. Appl Environ Microbiol 75:5445–5450. doi:10.1128/aem.00726-09
9. Rolleke S, Witte A, Wanner G, Lubitz W (1998) Medieval wall paintings - a habitat for archaea: identification of archaea by denaturing gradient gel electrophoresis (DGGE) of PCR-amplified gene fragments coding for 16S rRNA in a medieval wall painting. Int Biodeterioration Biodegradation 41:85–92. doi:10.1016/s0964-8305(98)80011-5
10. Rea S, Bowman JP, Popovski S, Pimm C, Wright ADG (2007) *Methanobrevibacter millerae* sp nov and *Methanobrevibacter olleyae* sp nov., methanogens from the ovine and bovine rumen that can utilize formate for growth. Int J Syst Evol Microbiol 57:450–456. doi:10.1099/ijs.0.63984-0
11. Miller TL, Lin CZ (2002) Description of *Methanobrevibacter gottschalkii* sp nov., *Methanobrevibacter thaueri* sp nov., *Methanobrevibacter woesei* sp nov and *Methanobrevibacter wolinii* sp nov. Int J Syst Evol Microbiol 52:819–822. doi:10.1099/ijs.0.02022-0
12. Sprenger WW, van Belzen MC, Rosenberg J, Hackstein JHP, Keltjens JT (2000) *Methanomicrococcus blatticola* gen. nov., sp

nov., a methanol- and methylamine-reducing methanogen from the hindgut of the cockroach *Periplaneta americana*. Int J Syst Evol Microbiol 50:1989–1999
13. Jarvis GN, Strompl C, Burgess DM, Skillman LC, Moore ERB, Joblin KN (2000) Isolation and identification of ruminal methanogens from grazing cattle. Curr Microbiol 40:327–332. doi:10.1007/s002849910065
14. Dridi B, Fardeau ML, Ollivier B, Raoult D, Drancourt M (2012) *Methanomassiliicoccus luminyensis* gen. nov., sp nov., a methanogenic archaeon isolated from human faeces. Int J Syst Evol Microbiol 62:1902–1907. doi:10.1099/ijs.0.033712-0
15. Kulik EM, Sandmeier H, Hinni K, Meyer J (2001) Identification of archaeal rDNA from subgingival dental plaque by PCR amplification and sequence analysis. FEMS Microbiol Lett 196:129–133. doi:10.1016/s0378-1097(01)00051-9
16. Kembel SW, Jones E, Kline J, Northcutt D, Stenson J, Womack AM, Bohannan BJM, Brown GZ, Green JL (2012) Architectural design influences the diversity and structure of the built environment microbiome. ISME 6:1469–1479. doi:10.1038/ismej.2011.211
17. Rintala H, Pitkaeranta M, Toivola M, Paulin L, Nevalainen A (2008) Diversity and seasonal dynamics of bacterial community in indoor environment. BMC Microbiol 8:1–13, doi:10.1186/1471-2180-8-56
18. Rintala H, Pitkaranta M, Taubel M (2012) Microbial communities associated with house dust. In: Laskin AI, Sariaslani S, Gadd GM (eds) Advances in Applied Microbiology, vol 78., pp 75–120
19. Pitkaranta M, Meklin T, Hyvarinen A, Paulin L, Auvinen P, Nevalainen A, Rintala H (2008) Analysis of fungal flora in indoor dust by ribosomal DNA sequence analysis, quantitative PCR, and culture. Appl Environ Microbiol 74:233–244. doi:10.1128/aem.00692-07
20. Amend AS, Seifert KA, Samson R, Bruns TD (2010) Indoor fungal composition is geographically patterned and more diverse in temperate zones than in the tropics. Proc Natl Acad Sci U S A 107:13748–13753. doi:10.1073/pnas.1000454107
21. Flannigan B (2011) Microorganisms in indoor environment. In: Flannigan B, Samson RA, Miller D (eds) Microorganisms in home and indoor work environments: diversity, health impacts, investigation and control. CRC Press, Boca Raton
22. Kuhn DM, Ghannoum MA (2003) Indoor mold, toxigenic fungi, and *Stachybotrys chartarum*: Infectious disease perspective. Clin Microbiol Rev 16:144–172. doi:10.1128/cmr.16.1.144-172.2003
23. Li DW, Kendrick B (1995) Indoor aeromycota in relation to residential characteristics and allergic symptoms. Mycopathologia 131:149–157. doi:10.1007/bf01102894
24. Li DW, Kendrick B (1996) Functional and causal relationships between indoor and outdoor airborne fungi. Can J Bot-Revue Canadienne De Botanique 74:194–209
25. Mentese S, Arisoy M, Rad AY, Gullu G (2009) Bacteria and fungi levels in various indoor and outdoor environments in Ankara, Turkey. Clean-Soil Air Water 37:487–493. doi:10.1002/clen.200800220
26. Samson RA (2011) Ecology and general characteristics of indoor fungi. In: Olaf C, Adan G, Samson RA (eds) Fundamentals of mold growth in indoor environments and strategies for healthy living. Wageningen Academic Publishers, Netherland, pp 101–107
27. Pakarinen J, Hyvarinen A, Salkinoja-Salonen M, Laitinen S, Nevalainen A, Makela MJ, Haahtela T, von Hertzen L (2008) Predominance of Gram-positive bacteria in house dust in the low-allergy risk Russian Karelia. Environ Microbiol 10:3317–3325. doi:10.1111/j.1462-2920.2008.01723.x
28. Yuan I, Xu JR, Millar BC, Dooley JSG, Rooney PJ, Alexander HD, Moore JE (2007) Molecular identification of environmental bacteria in indoor air in the domestic home: description of a new species of *Exiguobactetium*. Int J Environ Health Res 17:75–82. doi:10.1080/09603120601124199
29. Kembel SW, Meadow JF, O'Connor TK, Mhuireach G, Northcutt D, Kline J, Moriyama M, Brown GZ, Bohannan BJM, Green JL (2014) Architectural design drives the biogeography of indoor bacterial communities. PLoS One 9:1–10. doi:10.1371/journal.pone.0087093
30. Meadow JF, Altrichter AE, Kembel SW, Kline J, Mhuireach G, Moriyama M, Northcutt D, O'Connor TK, Womack AM, Brown GZ, Green JL, Bohannan BJM (2014) Indoor airborne bacterial communities are influenced by ventilation, occupancy, and outdoor air source. Indoor Air 24:41–48. doi:10.1111/ina.12047
31. Adams RI, Miletto M, Taylor JW, Bruns TD (2013) Dispersal in microbes: fungi in indoor air are dominated by outdoor air and show dispersal limitation at short distances. ISME 7:1262–1273. doi:10.1038/ismej.2013.28
32. Sousa ACA, Almeida J, Pereira CC, Pastorinho MR, Pereira AMC, Nogueira AJA, Taborda-Barata L, Teixeira JP, Correia ACM, Alves A (2014) Characterization of fungal communities in house dust samples collected from central Portugal—a preliminary survey. J Toxicol Environ Health A 77:972–982. doi:10.1080/15287394.2014.911137
33. Subbarao P, Anand SS, Becker AB, Befus AD, Brauer M, Brook JR, Denburg JA, HayGlass KT, Kobor MS, Kollmann TR, Kozyrskyj AL, Lou WY, Mandhane PJ, Miller GE, Moraes TJ, Pare PD, Scott JA, Takaro TK, Turvey SE, Duncan JM, Lefebvre DL, Sears MR, CHILD Study Investigators (2015) The Canadian Healthy Infant Longitudinal Development (CHILD) Study: examining developmental origins of allergy and asthma. Thorax 70:998–1000. doi:10.1136/thoraxjnl-2015-207246
34. Takaro TK, Scott JA, Allen RW, Anand SS, Becker AB, Befus AD, Brauer M, Duncan J, Lefebvre DL, Lou W, Mandhane PJ, McLean KE, Miller G, Sbihi H, Shu H, Subbarao P, Turvey SE, Wheeler AJ, Zeng L, Sears MR, Brook JR, CHILD Study investigators (2015) The Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort study: assessment of environmental exposures. J Expo Sci Environ Epidemiol 25:580–592. doi:10.1038/jes.2015.7
35. Pakpour S, Olishevska SV, Prasher SO, Milani AS, Chénier MR (2013) DNA extraction method selection for agricultural soil using TOPSIS multiple criteria decision-making model. Am J Mol Biol 3:215–228
36. Kemnitz D, Kolb S, Conrad R (2005) Phenotypic characterization of Rice Cluster III archaea without prior isolation by applying quantitative polymerase chain reaction to an enrichment culture. Environ Microbiol 7:553–565. doi:10.1111/j.1462-2920.2005.00723.x
37. Suzuki MT, Taylor LT, DeLong EF (2000) Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 5′-nuclease assays. Appl Environ Microbiol 66:4605–4614. doi:10.1128/aem.66.11.4605-4614.2000
38. Clarke KR, Gorley RN (2015) PRIMER v7: User Manual/Tutorial. PRIMER-E, Plymouth
39. StatSoft Inc. (2013) Electronic Statistics Textbook. StatSoft. WEB: http://www.statsoft.com/textbook/, Tulsa, OK.
40. Bintrim SB, Donohue TJ, Handelsman J, Roberts GP, Goodman RM (1997) Molecular phylogeny of archaea from soil. Proc Natl Acad Sci U S A 94:277–282. doi:10.1073/pnas.94.1.277
41. Buckley DH, Graber JR, Schmidt TM (1998) Phylogenetic analysis of nonthermophilic members of the kingdom Crenarchaeota and their diversity and abundance in soils. Appl Environ Microbiol 64:4333–4339
42. Ochsenreiter T, Selezi D, Quaiser A, Bonch-Osmolovskaya L, Schleper C (2003) Diversity and abundance of Crenarchaeota in terrestrial habitats studied by 16S RNA surveys and real time PCR. Environ Microbiol 5:787–797. doi:10.1046/j.1462-2920.2003.00476.x
43. Simon HM, Dodsworth JA, Goodman RM (2000) Crenarchaeota colonize terrestrial plant roots. Environ Microbiol 2:495–505. doi:10.1046/j.1462-2920.2000.00131.x

44. Saengkerdsub S, Ricke SC (2014) Ecology and characteristics of methanogenic Archaea in animals and humans. Crit Rev Microbiol 40:97–116. doi:10.3109/1040841x.2013.763220
45. Auguet JC, Barberan A, Casamayor EO (2010) Global ecological patterns in uncultured Archaea. ISME 4:182–190. doi:10.1038/ismej.2009.109
46. Bates ST, Berg-Lyons D, Caporaso JG, Walters WA, Knight R, Fierer N (2011) Examining the global distribution of dominant archaeal populations in soil. ISME 5:908–917. doi:10.1038/ismej.2010.171
47. Yin Q, Fu BB, Li BY, Shi XC, Inagaki F, Zhang XH (2013) Spatial variations in microbial community composition in surface seawater from the Ultra-Oligotrophic Center to rim of the South Pacific Gyre. PLoS One 8:1–12. doi:10.1371/journal.pone.0055148
48. Frohlich-Nowoisky J, Nespoli CR, Pickersgill DA, Galand PE, Muller-Germann I, Nunes T, Cardoso JG, Almeida SM, Pio C, Andreae MO, Conrad R, Poschl U, Despres VR (2014) Diversity and seasonal dynamics of airborne archaea. Biogeosciences 11:6067–6079. doi:10.5194/bg-11-6067-2014
49. Orell A, Frols S, Albers SV (2013) Archaeal biofilms: The great unexplored. Annu Rev Microbiol 67:337–354. doi:10.1146/annurev-micro-092412-155616
50. Frols S (2013) Archaeal biofilms: widespread and complex. Biochem Soc Trans 41:393–398. doi:10.1042/bst20120304