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

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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

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 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 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 m²), and if insufficient dust was obtained, the sampling area was expanded. Research technicians visually observed the thimbles after vacuuming 2 m²; 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′ CGGGGYGCASCAGGCAGGAA 3′) and A934bR (5′ GTGCTCCCCGGCCAATTCCT 3′) primers for archaea [36] and BACT1369F (5′ CGGTAATACGTTCGAGG 3′) and PROK1492R (5′ GGGTACTCGTTACGAGG 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 10⁶ copies/μL. Amplification efficiencies of 92.2–94.7 % (R² > 0.985) and 90.1–105.8 (R² > 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 STATISTICA 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

| Sub-sample of 668 collected environmental factors |
|--------------------------------------------------|
| Building design characteristics                  |
| Age of ceiling                                    |
| Age of floor                                      |
| Age of house                                      |
| Basement condition                                |
| Basement dampness                                 |
| Basement foundation                               |
| Child room area (sq. m)                           |
| Child room carpet area (sq. m)                    |
| Child room wall cover                             |
| Child room window cover                           |
| Cleanliness of basement                           |
| Condensation on bedroom                           |
| Windows in cooler weather                         |
| Evidence of leak in the house                     |
| Finished basement or added insulation             |
| Floor level                                       |
| Furnace age                                       |
| Furnace condition                                 |
| Most used room area (sq. m)                       |
| Most used room carpet area (sq. m)                |
| Number of rooms in the house                      |
| Presence of garage                                |
| Type of garage                                    |
| Presence of swimming pool                         |
| Presence of upgraded plumbing system              |
| Total volume of the house                         |
| Type of flooring                                  |
| Type of foundation                                |
| Type of fuel in the house                         |
| Type of furnace’s filter                          |
| Type of garage                                    |
| Type of house                                     |
| Type of insulation                                |
| Type of lawn                                      |
| Type of wall covering                             |
| Type and density of occupants                     |
| Number of adults in the house                     |
| Number of children in the house                   |
| Number of plants                                  |
| Frequency of bathroom fan usage                   |
| Frequency of house cleaning                       |
| Frequency of keeping the child bedroom’s window open|
| Frequency of keeping the most used room’s window open|
| Hanging clothes inside the house                  |
| Presence of stuff toys                            |
| Type of vacuum                                    |
| Usage level of gas fireplace                      |
| Usage level of radiators                          |
| Use of chemical spray and cloth                   |
| Use of garden sprays/weed killers                 |
| Use of mop                                        |
| Use of unscented or scented candles               |
| Use of antibacterial hand cleaner                 |
| Use of broom                                      |
| Use of chemical sprays for cleaning               |
| Use of disinfectants                              |
| Use of feather duster                             |
| Use of floor cleaners                             |
| Use of glass cleaners                             |
| Use of liquid or solid air fresheners             |
| Use of multi-surface cleaners                     |
| Use of oven cleaners                              |
| Use of plug-in deodorizers                        |
| Use of plumbing cleaners                          |
| Use of scented laundry detergents                 |
| Use of spray air fresheners                       |
| Use of toilet bowl cleaners                       |
| Use of vacuum                                     |
| Use of wet cloth (water only) for cleaning        |
| Use of swiffer wet jet                            |
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 $5.01 \pm 0.14$ (log 16S rRNA gene copies/g dust, mean±SE) in bedrooms and $5.58 \pm 0.13$ in the most used rooms. However, these magnitudes were notably lower than indoor bacteria, which were between $9.20 \pm 0.32$ in bedrooms and $9.17 \pm 0.32$ 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

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

|        | ARCHAEA          | BACTERIA         |
|--------|------------------|------------------|
| Bedroom| ![Graph](image)  | ![Graph](image)  |
| Most Used Room | ![Graph](image) | ![Graph](image)  |
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

### 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<sup>a</sup> | Bedroom | The most used room |
|-------------------------------------|---------|--------------------|
| **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**                     |         |                    |
| 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 |

<sup>a</sup> 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.

**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.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

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