The indoor environment -

a potent source for intact human-associated anaerobes

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Abstract

People in westernized countries spend most of their time indoors. As interaction and exchange of microbes between the human body and its environment is crucial for a healthy human microbiome, the built environment (BE) might represent a potent source for commensal microbes. Anaerobic microbes are especially of interest, as acquisition routes of oxygen-sensitive microbes such as obligate or facultative anaerobes to the human microbiome are not sufficiently clarified yet.

We sampled a total of ten bathroom floors and used propidium monoazide (PMA) to assess the viability of the collected prokaryotes. Quantitative and qualitative analysis revealed that most of the microbial taxa are of human origin. Less than 25% of the prokaryotic signatures originate from intact - and thus potentially living - cells with an apparent survival advantage for aerobic and stress resistant taxa. Although the dominant microbial fraction identified on the bathroom floors are aerobes, we could verify could verify the presence of strictly anaerobic taxa - including methanogenic archaea - in PMA-treated samples, indicating a limited, but substantial capability of those taxa to survive in the BE. As methanogens are regarded as highly sensitive to aerobic conditions, oxygen tolerance experiments were performed with human-associated isolates to validate their survival. We could confirm that human-associated methanogens are capable of surviving oxic conditions for at least 6h.

In conclusion, we collected strong evidence supporting the notion that obligate anaerobic taxa are capable of surviving in the BE for a limited amount of time and thus, making the BE a potential source of anaerobic human commensals.

Introduction

The development of a healthy human microbiome depends on extensive microbial transmission from other hosts, food, water, but also reservoirs in the built environment (BE; Browne et al., 2017). Especially for newborn infants, acquisition of microbes is a crucial process as they display an empty niche for a variety of microorganisms, differently to adults with usually well-established, complex and stable microbiomes. Until a fairly stable microbiome is reached within the third year after birth (Stewart et al., 2018), numerous microorganisms are acquired from other microbiomes. This maturation process (including its speed and quality) depends on various factors, including birth mode (Caesarean section vs. vaginal birth), type of feeding (breast-fed vs. formula-fed), medication (e.g. antibiotics), time point of introduction of solid food, quality of social interactions and contact to animals and nature (Guaraldi & Salvatori, 2012; Moossavi & Azad, 2020; Pasolli et al., 2019; Rocchi et al., 2020). During maturation of the intestinal microbiome, facultative anaerobic microorganisms, which initially thrive in the gastrointestinal tract (GIT) after birth, are subsequently replaced by obligate anaerobes, which support the optimal fermentation of solid food (Browne et al., 2017).

The abiotic BE together with its indoor microbiome (reviewed by Chase et al., 2016; Karen C. Dannemiller, 2019; Gilbert & Stephens, 2018; Horve et al., 2020; Kelley & Gilbert, 2013) is considered to have an important role in the development and maintenance of a healthy human microbiome (Browne et al., 2017). As nowadays, the population of urban areas of high-income countries spends on average 90% of the day indoors, the abiotic BE and its indoor microbiome represent one of the major reservoirs of environmental microbes (Klepeis et al., 2001). This reservoir does not only feed and shape the human microbiome early in life (see A. W. Brooks et al., 2018; B. Brooks et al., 2014), but also contributes to re-colonisation after disease and infection, or antibiotic treatment (Shan et al., 2019).

Cross-talk between the human being and the BE can imply both positive and negative aspects. On the one hand, under rare circumstances, indoor microbes could trigger immediate health issues, in particular in the course of pathogen and resistance transmission in hospital environments (Lax, Sangwan, et al., 2017; Mahnert et al., 2019; Mora et al., 2016). On the other hand, several studies point out beneficial effects of a healthy indoor microbiome, including the observation that the presence of animal-associated and farm-like indoor microorganisms of rural areas is linked to decreased asthma and allergy risk (K. C. Dannemiller et al., 2014; Ege et al., 2012; Kirjavainen et al., 2019).

For a successful transmission of microbes between hosts via the built environment, their survivability outside of their natural habitat ("ex-host" survivability) is essential (Browne et al., 2017; Costello et al., 2012). Next to a number of different stressors including UV radiation and desiccation (Browne et al., 2017), exposure to atmospheric oxygen is indeed most critical for the majority of gut commensals, as estimated 99% of all microorganisms thriving in the distal colon are obligate anaerobes (Eckburg et al., 2005). The extent of aero-tolerance among species is variable, and usually, strictly anaerobic taxa are unable to actively replicate outside of the gastrointestinal tract (GIT; Ley et al., 2008; Meehan & Beiko, 2014). To overcome this problem, numerous (anaerobic) bacteria protect themselves through sporulation, as spores provide the opportunity to escape difficult environmental situations for an extended period of time. It is considered that up to 30% of all intestinal microorganisms have the capability to form spores, including Lachnospiraceae, Ruminococcaceae, Clostridiaceae and Peptostreptococcaceae (Browne et al., 2017). After being transferred, spores react to trigger signals (such as bile acids) by germination, spreading and colonising in the new host thereafter (Browne et al., 2017).

Non-spore formers, however, must have other strategies to tolerate environmental stresses. Usually, strictly anaerobic microorganisms, such as *Roseburia*, die within few minutes when exposed to atmospheric oxygen, as they have not evolved mechanisms to avoid and repair the damage caused by reactive oxygen species (ROS) through catalases, peroxidases or

superoxide dismutases (Miller & Britigan, 1997; Rolfe et al., 1978). Thus, for most of the strictly anaerobic microbiome members, including methanogenic archaea, the survival strategies and transmission routes are largely unknown.

Methanogenic archaea ("methanogens") are, besides bacteria, viruses and small eukaryotes, important compounds of the human GIT (Maria Chibani et al., 2020). They are obligate anaerobic microorganisms, without the capability to form spores. Despite their oxygen sensitivity, the most common representative, *Methanobrevibacter smithii*, can be detected in almost 96% of the adult population (Dridi et al., 2009), while being rarely found in younger cohorts (Palmer et al., 2007), especially in children born via Caesarean section (Wampach et al., 2017).

In this communication, we address the major question, whether the BE could be a potent reservoir for anaerobic, commensal microorganisms. For this, we analysed the indoor microbiome of ten households and put the microbiome information into context of human microbiome information. As the bathroom represents the richest source of GIT-derived microorganisms (Browne et al., 2017; Dunn et al., 2013), we sampled surfaces in the vicinity of toilets from different households. A major shortcoming of previous studies was the lack of distinction between viable microorganisms (with the potential to colonise a new host) and non-viable microorganisms (Browne et al., 2017); this shortcoming was herein addressed by using propidium monoazide (PMA) treatment combined with high-throughput 16S rRNA gene sequencing, by which we were able to identify signatures of intact and thus probably living microorganisms. As archaeal cells were frequently detected amongst the surviving proportion of the BE microbiome, we analysed the BE archaeome in more detail and were able to show that human-associated methanogens can survive air exposure and could thus be transferred to another human microbiome through the BE.

Material and methods

Sample collection and DNA extraction

Samples were collected from ten different family houses in the vicinity of Graz, Austria (in March 2017). All houses were occupied by at least one adult and five of the houses by families with children. The sampling surface was selected by considering the richest source of GIT-derived microorganisms (Dunn et al., 2013), namely the bathroom. Per household, two areas of 30 cm^2 in the proximity of the toilet (example shown in Supplementary Fig. 1a) were cleaned with bleach and sterile water and left uncovered and untouched for 7 days. After 7 days, the areas were sampled using a sterile nylon swab (FLOQSwabsTM, Copan, Brescia, Italy) that has been dipped in 0.9% saline (NaCl) solution. Each area was sampled with the swab three times, by rotating the swab every time before sampling the area again (Supplementary Fig. 1b).

The samples were transported back to the laboratory on ice packs on the day of the sampling. One of the two samples from each house, which were collected in parallel, was treated with PMA (propidium monoazide) as previously described (Mora et al., 2016) to differentiate between cells with an intact membrane. PMA is a photo-reactive DNA binding dye which intercalates with free DNA inhibiting downstream DNA amplification thereof. As PMA is not permeable for intact cell walls, a molecular distinction between PMA-masked free DNA from disrupted, most probably dead cells, and DNA from intact, most probably living cells can be made (Nocker et al., 2007). In the following, we use the term “PMA” to indicate samples treated with PMA (= intact cells only), and the term “nonPMA” for samples not treated with PMA (= both, intact and dead cells/free DNA).

For genomic DNA extraction, the indoor samples were processed using the FastDNA Spin Kit (MP Biomedicals, Germany) according to manufacturer's instructions. The genomic DNA

was eluted in 100 µL elution buffer, and the concentration was measured using Qubit™ dsDNA HS Assay Kit (ThermoFisher Scientific).

PCR and qPCR

The obtained genomic DNA was used to amplify the V4 region of the 16S rRNA gene using Illumina-tagged primers 515F and 806R (Supplementary Tab. 1). In order to identify the archaeal communities present in the samples, a nested PCR was performed using the primer combination 344F-1041R/Illu519F-Illu806R as described previously (Pausan et al., 2019). The PCR reaction was performed in a final volume of 25 µL containing: TAKARA Ex Taq® buffer with $MgCl_2$ (10 X; Takara Bio Inc., Tokyo, Japan), primers 200 nM, dNTP mix 200 µM, TAKARA Ex Taq® Polymerase 0.5 U, water (Lichrosolv®; Merck, Darmstadt, Germany), and DNA template (1-2 µL of genomic DNA). The PCR amplification conditions are listed in Supplementary Tab. 2.

The bacterial and archaeal 16S rRNA gene copies were determined using SYBR based qPCR with the primer pair Bac331F-Bac797R and A806f-A958r, respectively (Supplementary Tab. 1). The reaction mix contained: 1x SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad, Hercules, USA), 300 nM of forward and reverse primer, 1 µL gDNA template, PCR grade water (Lichrosolv®; Merck, Darmstadt, Germany). The qPCR was performed in triplicates using the CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, USA). The qPCR conditions are given in Supplementary Tab. 2. Crossing point (Cp) values were determined using the regression method within the Bio-Rad CFX Manager software version 3.1. Absolute copy numbers of bacterial 16S rRNA genes were calculated using the Cp values and the reaction efficiencies based on standard curves obtained from defined DNA samples from *Escherichia coli* and *Nitrososphaera viennensis* (Probst et al., 2013). The qPCR efficiency was between 85-105%, and the R^2 values were

always above 0.9. Detection limits were defined based on the average Cp values of non-template controls (triplicates) and the corresponding standard curves of the positive controls.

NGS-based 16S rRNA gene sequencing

Library preparation and sequencing of the amplicons were carried out at the Core Facility Molecular Biology, Center for Medical Research at the Medical University Graz, Austria. In brief, DNA concentrations were normalised using a SequalPrep™ normalisation plate (Invitrogen), and each sample was indexed with a unique barcode sequence (8 cycles index PCR). After pooling of the indexed samples, a gel cut was carried out to purify the products of the index PCR. Sequencing was performed using the Illumina MiSeq device and MS-102-3003 MiSeq® Reagent Kit v3-600cycles (2x250 cycles). The obtained fastq data is available in the European Nucleotide Archive under the study accession number: PRJEB31915.

The fastq data analysis was performed using QIIME2 (Bolyen et al., 2019) as described previously (Mahnert et al., 2020). After quality filtering, the DADA2 algorithm (Callahan et al., 2016) was used to denoise truncated reads and generate amplicon sequence variants (ASVs). Taxonomic classification (Bokulich et al., 2018) was based on the SILVA v132 database (Quast et al., 2013) and the obtained feature table and taxonomy file were used for further analysis (Supplementary Tab. 3). The overlapping features from negative controls (DNA extraction and PCR negative controls) were manually subtracted or removed from both the bacterial and archaeal dataset. The reads classified as chloroplast and mitochondria were also removed.

Human microbiome data

In order to assess to which extent the microbial community indoors is affected and affects the human microbiome, a representative dataset of the human microbiome from several body sites was collected. We took advantage of several previous in-house projects, covering the microbiome of skin, nasal cavity, saliva, urine, vagina, and stool (for more details, please

see: Supplementary Tab. 4). All samples were taken from healthy participants and processed in our lab with methods similarly to the current study: DNA was extracted by a combination of enzymatic and mechanical lysis; amplification of the 16S rRNA gene was done using the "universal" primers based on Caporaso et al. (2011) or Walters et al. (2016) and library preparation, as well as sequencing, were performed by the Core Facility Molecular Biology at the Medical University Graz, Austria. Obtained raw reads were processed in parallel to the indoor microbiome data of the current study (Supplementary Tab. 5 and 8).

Data analysis and statistics

In general, data analysis of the microbial data is based on the indoor data alone (Supplementary Tab. 3 and 8). Comparative analysis between indoor and human microbiomes are based on the mixed dataset (Supplementary Tab. 5 and 9). Bar charts, box plots, and bubble plots are based on relative abundances and were constructed for both the bacterial and archaeal communities at different taxonomic levels using the *phyloseq* (McMurdie & Holmes, 2013) and *ggplot2* package in R (Wickham, 2016). Significant differences between microbial taxa were calculated in R using Wilcoxon Signed-Rank Tests for dependent samples (e.g. PMA and nonPMA) or Mann–Whitney U test for independent samples (e.g. indoor and human). P values were adjusted for multiple testing using the method after Benjamini & Hochberg (1995). The online-tool Calypso (Zakrzewski et al., 2017) was used to calculate alpha diversity metrics, Linear discriminant analysis Effect Size (LEfSe), the bar plots and principal coordinates analysis (PCoA) plots based on Bray-Curtis. Before analysis in Calypso, the data was normalised using total sum normalisation (TSS) combined with square root transformation. For alpha diversity, reads were rarefied to 5,588 and analysed based on Shannon and richness indices. To test for differences in the beta diversity between sample categories, PERMANOVA analysis was performed in QIIME2 based on Bray-Curtis distances. BugBase (Ward et al., 2017) was used to predict potential phenotypes such as aerobic, anaerobic, facultative anaerobic and stress-tolerant

communities which were present in the PMA and nonPMA treated samples. SourceTracker2 analysis (Knights et al., 2011) was performed with default settings. Sampling depth was set to 1,000 reads for source and sink, respectively.

A phylogenetic tree was constructed to determine if the sequences belonging to methanogens identified in the analysed samples are of human origin. All sequences classified within the genera *Methanobacterium*, *Methanobrevibacter* and *Methanomassiliicoccus* from our analysed samples were used for creating the phylogenetic tree. Additionally, 16S rRNA sequences of species of *Methanobacterium*, *Methanobrevibacter* and *Methanomassiliicoccus* previously identified in the human body or the environment were included from NCBI and two other studies (Borrel et al., 2017; Pasolli et al., 2019). The alignment was performed using the SILVA SINA alignment tool (Pruesse et al., 2012). The sequences were cropped to the same length using BioEdit and used afterwards to construct a tree based on the maximum-likelihood algorithm with a bootstrap value of 1,000 using MEGA7 (Kumar et al., 2016). The phylogenetic tree was further processed using the online tool iTOL (Letunic & Bork, 2007).

Definition of obligate anaerobic taxa

Bacterial and archaeal taxa were annotated as obligate anaerobic on family and genus level and marked with an asterix *. The categorisation is based on the “list of prokaryotes according to their aerotolerant or obligate anaerobic metabolism” V1.3 (Million & Raoult, 2018) and literature review.

Cultivation and oxygen tolerance test

An oxygen sensitivity test was implemented to determine if methanogenic archaeal strains can survive under aerobic conditions for a certain period of time. Three human-associated methanogenic strains (*Methanosphaera stadtmanae* DSM no. 3091, *Methanobrevibacter smithii* DSM no. 2375, and *Methanomassiliicoccus luminyensis* DSM no. 25720) and a newly

isolated strain (*Methanobrevibacter sp*., unpublished) have been tested for their ability to survive under aerobic conditions. The human-associated methanogen cultures were obtained from DSMZ and both, *M. stadtmanae* and *M. luminyensis* were grown in MPT1 medium at 37 °C (Mauerhofer et al., unpublished). The new *Methanobrevibacter* strain was isolated in our lab from a fresh stool sample using ATCC medium: 1340 MS medium for methanogens (https://www.atcc.org/). Both *Methanobrevibacter* strains were grown in MS medium at 37°C.

Cultures were used after 3-7 days of growth as follows: 1 mL culture was transferred to sterile 1.5 mL Eppendorf tubes and centrifuged at 10,000 x g for 10 min, the cell pellet was washed twice either anaerobically for the controls or aerobically for the oxygen exposed microorganisms with 1 mL sterile 1x PBS and centrifuged again at 10,000 x g for 10 min. After removing most of the PBS, the cell pellet was resuspended in the remaining liquid and exposed to aerobic or anaerobic conditions for different time points: 0h (30 minutes after transfer), 6h, 24h, 48h, and 168h (7 days). After exposure, the liquid from the tubes was transferred to a sterile growth medium in Hungate tubes and the cultures were left to grow for 2-3 weeks. Then, the first OD measurements (600 nm) and methane measurements were done using a spectrometer and methane sensor (BCP-CH4 sensor, BlueSens). For the methane measurement, 10 mL of gas-phase was taken from the Hungate tubes using an air-tight glass syringe. For each experiment, two sterile unopened Hungate tubes with media served as control, and two inoculated Hungate tubes with 0.5 mL of the culture served as a positive control. In addition, a tube of 1x PBS served as a control for 168h. Each experiment was performed in duplicates. After OD and methane measurements, microscopic observations were performed to determine the shape of the microorganisms growing in the medium. Oxygen saturation of the PBS was monitored in a control experiment using the FireStingO2 optical oxygen meter (PyroScience GmbH) and the oxygen probe OXROB10. Using the identical methodology as described above we were able to confirm an oxygen

saturation > 80% at any given time point (0h, 10 min, 20 min, 30 min, 45 min, 1 h, 2 h, 4 h, 6 h and 24 h) for every strain.

Results

In the following, we present information on the anaerobic microbial communities detected in bathrooms of family houses. We were interested to analyse whether the indoor environment is a potential source of anaerobic, commensal microorganisms like methanogens and could serve as a source to colonise the human body. For this purpose, we collected samples from two surface areas of ~30 cm^2 from bathrooms of ten households.

One of the two samples collected from the BE was treated with PMA, in order to mask the DNA of disrupted cells (Nocker et al., 2007). Comparing nonPMA (untreated) and PMA-treated samples allowed us to distinguish the overall (nonPMA) microbiome from the microbiome which is intact, and thus probably alive (PMA). Thus, we could draw conclusions on the survival of strictly anaerobic microorganisms under exposed conditions, which were further supported by experimental studies.

Although we present information on all detected microbial signatures, we focus specifically on strictly anaerobic taxa. For convenience, we highlighted taxa that mainly consist of strict anaerobes with an asterisk *.

The human microbiome is the predominant source of microbes in the BE

By using a universal 16S rRNA gene sequencing approach, overall more than 380,000 sequences were obtained (Bacteria: 99.86%, Archaea: 0.14%), corresponding to 3,684 ASVs and an average of 473 ± 110 ASVs per household (285 ± 50 and 245 ± 80 ASVs in nonPMA and PMA samples, respectively; Supplementary Tab. 3). We observed a high heterogeneity amongst households, with only 26 shared ASVs (0.7%) amongst all, and 351 ASVs (9.5%) shared between at least two households (Supplementary Tab. 6). The 26 ASVs present in all samples include typical skin-associated taxa such as *Staphylococcus*

(11 ASVs) and *Corynebacterium* (8 ASVs), but also *Finegoldia** (6 ASVs) and *Peptoniphilus** (1 ASV).

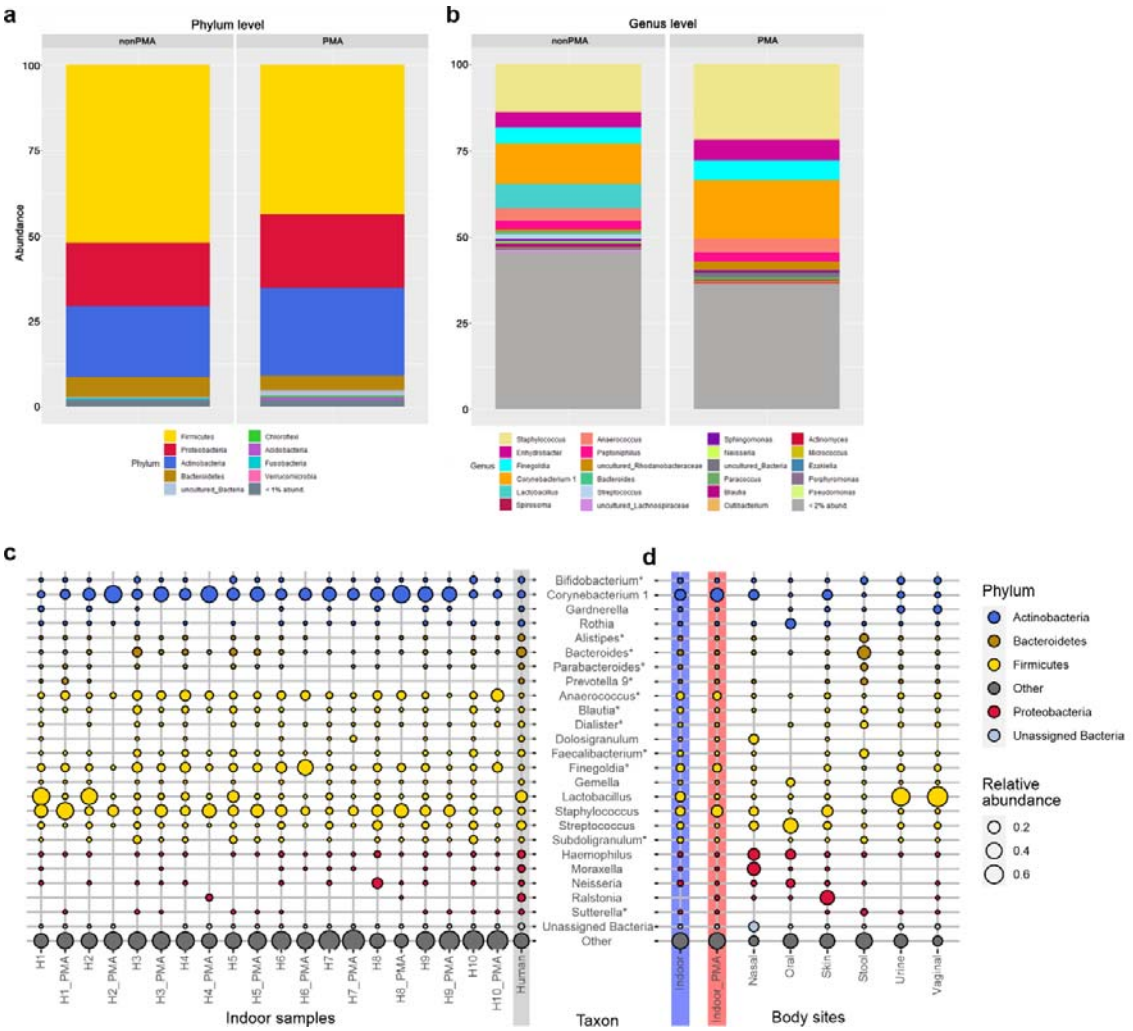


Fig. 1: Distribution and relative abundance of bacterial taxa in samples from different households/ bathrooms. The bar charts show the microbial composition of nonPMA and PMA treated bathroom floor samples at **(a)** phylum and **(b)** genus level. Genera with < 2% rel. abundance are summarised in grey. Bubble plots display the 25 most abundant genera (bubble size reflect their relative abundance): **(c)** Household samples (H1-H10) are depicted individually for nonPMA and PMA treatment together with human samples (grey background) that represent reads from several body sites including nasal cavity, skin, vagina, urine, stool, and oral samples. **(d)** Human samples in relation to nonPMA (blue background) and PMA (red background) bathroom samples. Genera are coloured according to their taxonomic phylum and taxa that predominantly contain strict anaerobes are marked by an asterisk.

A total of 32 phyla were observed and most sequences were classified to four phyla: Firmicutes (52.2% nonPMA, 41.2% PMA), Actinobacteria (20.3% nonPMA, 26.0% PMA),

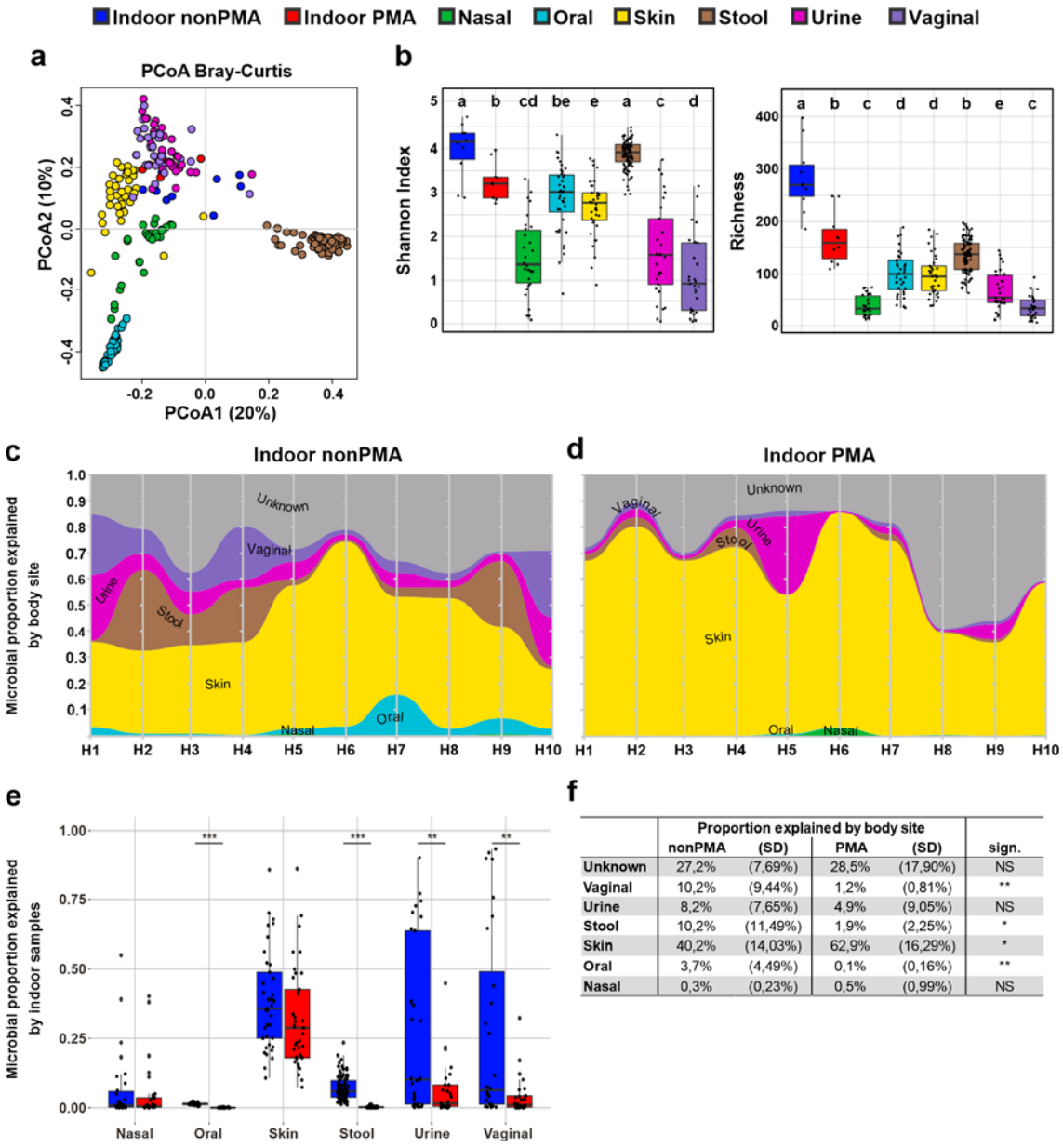
Proteobacteria (18.8% nonPMA, 23.3% PMA), and Bacteroidetes (6% nonPMA, 4.3% PMA) (Fig. 1a). None of those phyla changed significantly in abundance upon PMA treatment. According to Linear discriminant analysis Effect Size (LEfSe) and Wilcoxon signed-rank test, significant differences were only observed in four low-abundant phyla that showed an increase (Actinobacteria) or decrease (Tenericutes, Fusobacteria, Epsilonbacteraeota) in relative abundance upon PMA treatment ($P < 0.05$, Supplementary Fig. 2).

Genera associated with human skin (e.g. *Staphylococcus, Corynebacterium, Streptococcus, Neisseria, Micrococcus, Cutibacterium*), gastrointestinal and genitourinary tract (e.g. *Lactobacillus, Finegoldia*, Bacteroides*, Anaerococcus*, Peptoniphilus**, Lachnospiraceae*) have been identified in both nonPMA and PMA samples (Fig. 1c). Notably, *Bifidobacterium*, Rothia, Bacteroides*, Blautia*, Dialister*, Faecalibacterium*, Gemella, Subdoligranulum**, and *Haemophilus* were detected in every single household, but not in all PMA samples, indicating a potential impairment by environmental stressors.

Due to the high proportion of common human-associated commensals, we put the BE microbiome information into context with human microbiome information that was collected from other studies in our lab (see material and methods and Supplementary Tab. 4). Bubble plot analysis of the most abundant genera (Fig. 1d) revealed a specific pattern with respect to abundance in BE samples or human samples, retrieved from the nasal and oral cavity, skin, stool and vaginal samples. In particular, we observed, that signatures of typical human commensals such as *Corynebacterium*, *Anaerococcus**, *Dolosigranulum*, *Finegoldia**, *Gardnerella* and *Staphylococcus* were highly enriched in nonPMA indoor samples compared to human samples ($P_{adj} < 0.001$; Mann–Whitney U test), indicating an efficient transfer to indoor surfaces.

With respect to alpha diversity, nonPMA-indoor samples showed high Shannon indices, comparable to stool samples and even exceeded stool samples in richness more than two-fold (mean richness: 282 and 136 for nonPMA-indoor and stool, respectively; see Fig. 2).

Beta diversity analysis confirmed a separate grouping of all analysed body sites ($P_{adj} < 0.01$, PERMANOVA), except for urine and vaginal samples ($P_{adj} = 0.81$; Fig. 2a). However, we could not observe a grouping of indoor samples with a specific human body site as all of them cluster significantly differently to another ($P_{adj} < 0.01$).



	Proportion explained by body site				
	nonPMA	(SD)	PMA	(SD)	sign.
Unknown	27,2%	(7,69%)	28,5%	(17,90%)	NS
Vaginal	10,2%	(9,44%)	1,2%	(0,81%)	**
Urine	8,2%	(7,65%)	4,9%	(9,05%)	NS
Stool	10,2%	(11,49%)	1,9%	(2,25%)	*
Skin	40,2%	(14,03%)	62,9%	(16,29%)	*
Oral	3,7%	(4,49%)	0,1%	(0,16%)	**
Nasal	0,3%	(0,23%)	0,5%	(0,99%)	NS

Fig. 2 The indoor and human microbiome resemble another. (a) Principal coordinates analysis plots based on Bray-Curtis dissimilarity and **(b)** Alpha diversity indices (Shannon, left; Richness right) are depicted for all indoor samples (blue = untreated, red = PMA treated) together with representative human body site samples (green = nasal cavity, light blue = oral, yellow = skin, brown = stool, pink = urine, purple = vaginal). Significant differences are indicated by different letters above bars, defined by Mann–Whitney U test; $P < 0.05$, FDR adjusted (samples that share the same letter are not significantly different to another). The proportion of microbes

in 10 different bathrooms (H1 – H10) for **(c)** nonPMA and **(d)** PMA samples that can be explained by human body sites or are of unknown origin (grey). Each household is represented by a vertical bar, and values were fitted to 100%. **(e)** shows the proportion of microbes on different body sites that can be potentially explained by the bathroom microbiome; significant differences between treatments were defined by Kruskal-Wallis test; **, $P < 0.01$; ***, $P < 0.001$. **(f)** The table shows mean values and standard deviation of (d) and (e); significant differences between treatments were defined by Wilcoxon signed-rank test; NS, not significant; *, $P < 0.05$; **, $P < 0.01$.

In order to determine the level of impact of the human microbiome on our indoor samples, we performed SourceTracking 2 analysis (Knights et al., 2011). Independent from PMA treatment, about 75% of the indoor microbiome was associated with human sources. This identifies the human body as the dominant source for microbes detected in bathroom samples (Fig. 3a,b). Nevertheless, depending on the household, we observed large fluctuations in the proportion of human-associated taxa. Especially in PMA samples, this proportion ranges from 41 to 89%, probably indicating large variation in frequency and duration of exposure to a human being.

Upon PMA treatment, we observe a significant reduction of vaginal, stool, and oral signatures in the surface samples, while the proportion of microbes from human skin significantly increased ($P < 0.05$, Wilcoxon signed-rank test; see Fig. 3d). This indicates that skin-related microbes possess the highest capability for surviving in the bathroom environment.

Vice versa, microbial traces from the indoor environment could potentially serve as a source for the human microbiome, too (Fig. 3c). In agreement with other studies, the highest level of exchange between indoor and human microbes was identified for skin, sharing on average 29% and 32% of the microbial signatures with nonPMA and PMA samples from the bathroom. The overlap with other body sites was generally very low, and especially PMA treated samples shared on average only about 6% of the taxa with urine, 5% with nasal cavity, 4% with vaginal, and < 0.01% with oral and stool samples. Therefore, the impact of

the bathroom microbiome to human body sites except the skin is perceived to be very small or even neglectable.

Oxygen-tolerance is the main denominator for microbial survival indoors

As human commensals face several stressors in the BE many of them are perceived to be incapable of surviving in this environment for extended durations. We performed PMA treatment to mask DNA from disrupted cells, and in fact, the absolute abundance was significantly affected by PMA treatment (Fig. 3a). Results of the quantitative PCR revealed a 4.2-fold decrease in microbial 16S rRNA gene copy number (Fig. 4c), indicating that less than 25% of all microbial signatures originate from intact, probably living cells. This is also reflected by a significant drop in both alpha diversity parameters in PMA samples compared to untreated indoor samples (Shannon: $P_{adj} = 0.007$, richness: $P_{adj} = 0.0006$; Wilcoxon rank-sum tests, Fig. 2b). PCoA plots based on Bray-Curtis distances also showed a significant separation based on the PMA treatment ($P_{adj} = 0.018$) even though indoor samples were more similar to another compared to any human body site (Fig. 2a).

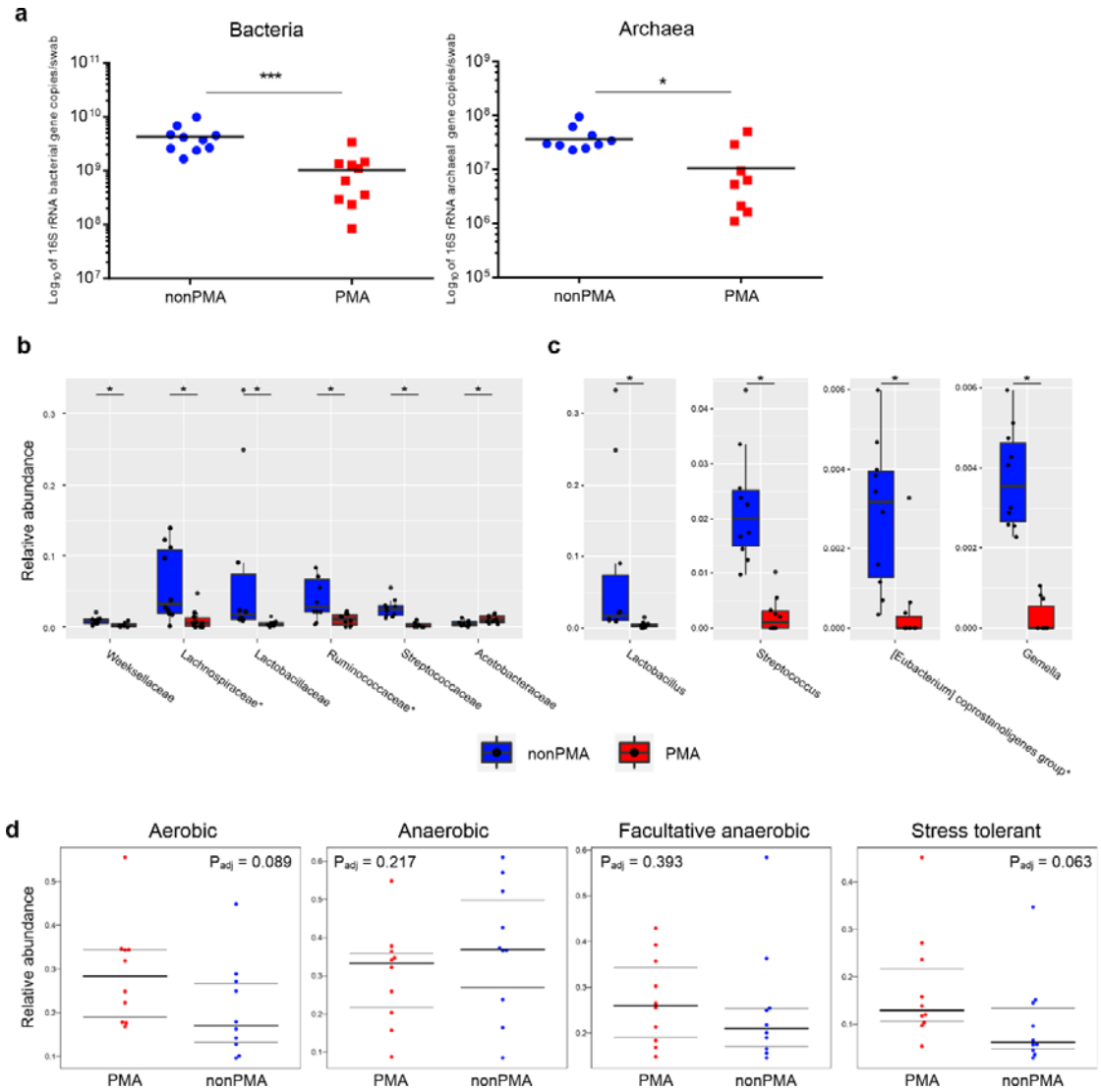


Fig. 3 PMA treatment affects prokaryotic abundance and composition. (a) Quantitative PCR analysis of untreated and PMA-treated bathroom floor samples using the universal and archaeal primer pairs. **(b)** Wilcoxon signed-rank test analysis of the relative abundance of the 50 and 100 most abundant bacterial **(b)** families and **(c)** genera, respectively, that were significantly affected by PMA treatment. Strict anaerobic taxa are marked by an asterix. Sign.: FDR adjusted Wilcoxon signed-rank test; *, $P_{adj} < 0.05$; ***, $P_{adj} < 0.01$. **(d)** The relative abundance of different phenotypes as determined through BugBase. Significances were automatically defined by Mann-Whitney U test in BugBase.

LEfSe was performed on PMA and nonPMA samples to determine which taxa are indicative for PMA samples and thus likely to survive in the indoor environment (Supplementary Fig. 3). Representative taxa for PMA were found to be predominantly environmental and aerobic (or aerotolerant) groups such as *Acidobacteria*, *Micromonosporales*, *Solirubrobacterales*, *Rhizobiales*, *Nocardiaceae*, *Bacillus*, and *Sphingomonas,* but also included human skin

commensals such as *Corynebacterium*_1, *Staphylococcus* or *Rombustia**. On the other hand, representative taxa for nonPMA samples were more diverse in their physiological traits and origin. They predominantly included (facultatively) anaerobic, non-spore forming, human-associated groups such as: *Bacteroidales**, *Lactobacillaceae*, multiple genera within the families *Lachnospiraceae** and *Ruminococcaceae**, *Bifidobacteriaceae**, as well as Christensenellaceae*, Veillonellaceae*, *Haemophilus*, *Neisseria* and the *Eubacterium coprostanoligenes* group*. Interestingly, some opportunistic pathogens (*Atopobium*, *Rothia* and *Campylobacter*) were also characteristic for, and significantly enriched in nonPMA samples (Supplementary Fig. 4), which suggests that those taxa might not survive on BE surfaces for extended periods of time.

After PMA treatment, we observed a significant change in relative abundance for 53 taxa among the most abundant bacterial genera and families ($P < 0.05$, $n_{genus} = 100$, $n_{family} = 50$, Wilcoxon signed-rank test, Supplementary Tab. 7), while only 10 taxa remained significant after adjustment for multiple testing ($P_{adj} < 0.05$, Fig. 3b,c). In concordance with our LEfSe analysis, a strong reduction was seen for non-spore forming, (facultative) anaerobic human commensals, particularly of *Lactobacillus*, *Streptococcus*, *Gemella* and the *Eubacterium coprostanoligenes* group*. But also *Ruminococcaceae* and several genera within this family were highly affected by PMA-treatment and seemed to be susceptible to stressors in the BE, whereas taxa within the family *Acetobacteraceae*, and other commensals such as *Bacillus* and *Staphylococcus* appear to be extraordinarily well suited for survival under BE conditions.

BugBase (Ward et al., 2017) was used to validate the observed phenotypes for nonPMA and PMA samples (Fig. 3d). In comparison with nonPMA samples, the PMA samples showed an increase in the relative abundance of aerobic ($P_{adj} = 0.089$) and facultative anaerobic communities ($P_{adj} = 0.393$), and a slight decrease in anaerobic communities ($P_{adj} = 0.217$) (Fig. 3d). Interestingly, stress tolerant phenotypes were increased in the PMA samples ($P_{adj} = 0.063$), indicating that the microorganisms surviving in the indoor environment might be better equipped to deal with BE-associated stresses.

Archaea are an integral component of the BE

An archaea-centric approach (Pausan et al., 2019) was used to explore the archaeal communities present on the sampled surfaces. Overall, we obtained about 376,000 archaeal sequences in both, PMA and nonPMA samples corresponding to 373 ASVs (on average 70.2 ± 22.8 ASVs/ house; 53.6 ± 16.8 ASVs/ nonPMA samples; 35.9 ± 22.1 ASVs/ PMA samples; Supplementary Tab. 8). The archaeal ASVs were classified into four different phyla, namely Euryarchaeota (36.6% relative abundance in nonPMA samples, PMA: 35.0%), Thaumarchaeota (nonPMA: 62.2%, PMA: 64.1%), Crenarcheota (nonPMA: 0.8%, PMA: 0.5%), and Nanoarchaeota (nonPMA: 0.4%, PMA: 0.5%) (Fig. 4a).

On family level, most reads were classified within the *Nitrososphaeraceae* (nonPMA: 62.1%, PMA: 64.1%), which is also the only archaeal family found in every household in this study (Fig. 4c). Among the *Nitrososphaeraceae*, the dominant genus identified was *Candidatus Nitrosocosmicus* (nonPMA: 22.5%, PMA: 15.9%), but sequences classified within *Candidatus Nitrososphaera* (nonPMA: 0.9%, PMA: 0.2%) were also identified (Fig. 4b). The second most abundant family was *Halococcaceae* (nonPMA: 19.7%, PMA: 28.7%), with *Halococcus* (nonPMA: 13.7%, PMA: 28.6%) as predominant genus. Interestingly, oxygen-sensitive methanogenic taxa were also detected in every household, but at low relative abundance (*Methanobacteria** nonPMA: 3.7%, PMA: 3.5%; *Methanomicrobia** nonPMA: 4.0%, PMA: 2.6%), with *Methanosarcina** (non-PMA: 3.2%, PMA: 1.8%), *Methanobrevibacter** (nonPMA: 2.4%, PMA: 0.2%), *Methanobacterium** (nonPMA: 1.3%, PMA: 3.3%), and *Methanomassiliicoccus** (nonPMA: 0.4%, PMA: 0.2%) being the most abundant genera.

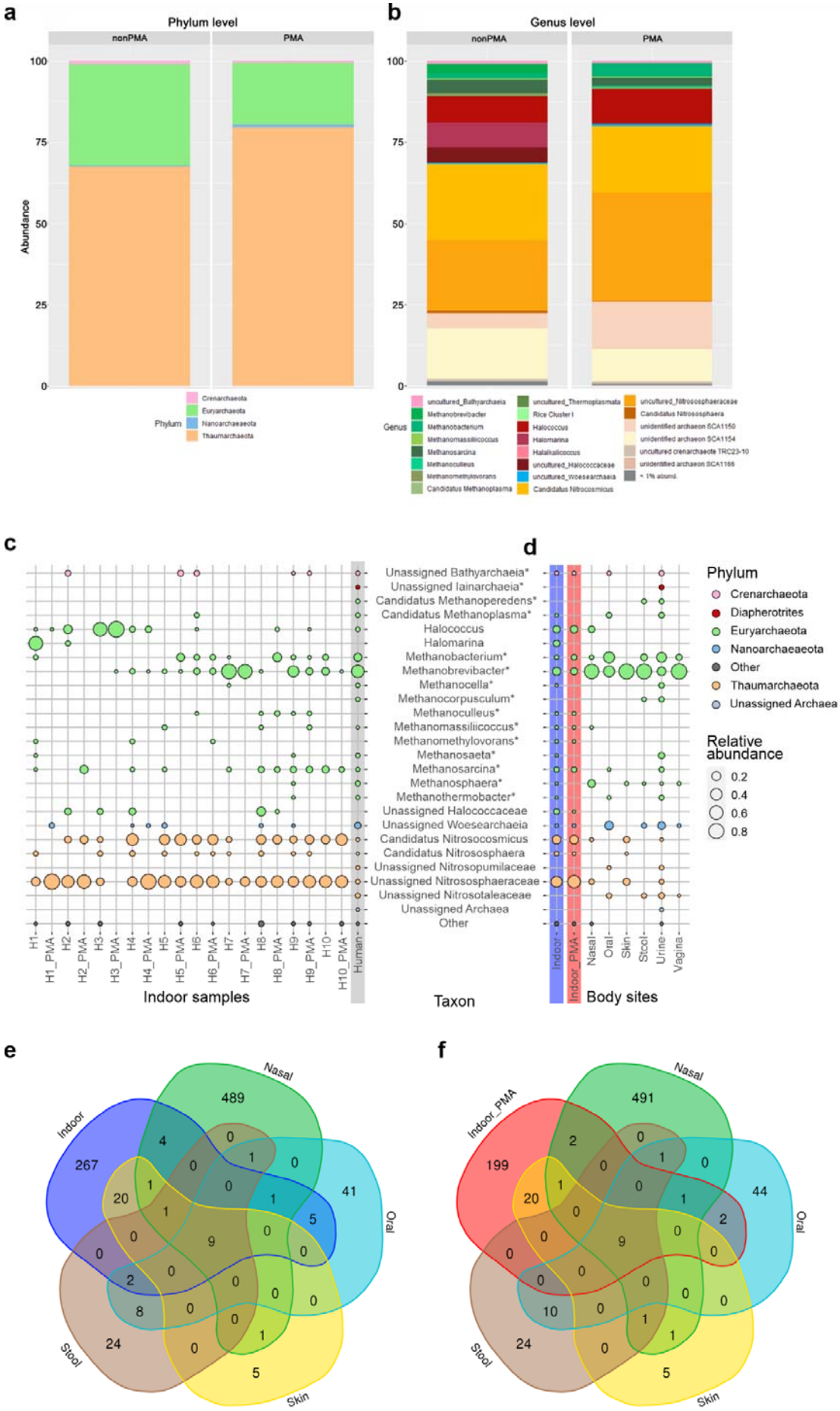

a
Phylum level
nonPMA
PMA
Abundance
Phylum
Crenarchaeota
Euryarchaeota
Nanoarchaeaeota
Thaumarchaeota
b
Genus level
nonPMA
PMA
Genus
uncultured_Bathyarchaeia
Methanobrevibacter
Methanobacterium
Methanomassiliicoccus
Methanosarcina
Methanoculleus
Methanomethylovorans
Candidatus Methanoplasma
uncultured_Thermoplasmata
Rice Cluster I
Halococcus
Halomarina
Halalkalicoccus
uncultured_Halococcaceae
uncultured_Woesearchaeia
Candidatus Nitrocosmicus
uncultured_Nitrososphaeraceae
Candidatus Nitrososphaera
unidentified archaeon SCA1150
unidentified archaeon SCA1154
uncultured crenarchaeote TRC23-10
unidentified archaeon SCA1166
< 1% abund.
c
Indoor samples
Taxon
d
Body sites
Phylum
Crenarchaeota
Diapherotrites
Euryarchaeota
Nanoarchaeaeota
Other
Thaumarchaeota
Unassigned Archaea
Relative abundance
0.2
0.4
0.6
0.8
e
Nasal
Indoor
Oral
Stool
Skin
f
Nasal
Indoor_PMA
Oral
Stool
Skin

Fig. 4. Archaeal community composition in indoor samples. Relative abundance of archaeal taxa among 16S rRNA reads from bathroom floor surfaces at **(a)** phylum and **(b)** genus level. Bubble plot showing relative abundances of the 25 most abundant archaeal genera found among bathroom and human samples: **(c)** Household samples (H1-H10) are depicted individually for nonPMA and PMA treatment with human samples (grey background) that represent reads from several body sites including nasal cavity, skin, vagina, urine, stool, and oral samples. **(d)** Bubble plots of the 25 most abundant archaeal genera, and displayed according to their original sample. Relative abundance is reflected by the size of the bubbles. Genera are coloured according to their taxonomic phylum and taxa that predominantly contain strict anaerobes are marked by an asterisk. Venn diagram of shared ASVs in **(e)** nonPMA indoor and **(f)** PMA indoor samples compared to nasal, oral, skin and stool samples (body sites that show the highest amount of shared ASVs).

Most archaeal taxa of the BE are likely of human origin

The archaeal community composition indicates that some of the archaeal signatures identified on the bathroom surface could be of human origin, as *Thaumarchaeota*, *Haloarchaea* and methanogens have been previously linked to the human body (Borrel et al., 2020). Thus, we analysed the data obtained from these surfaces together with data obtained through the same archaea centric approach from samples collected from the human body, namely stool (n = 38), urine (n = 43), nasal (n = 30), oral (n = 26), vaginal (n = 16) and skin (n = 7) samples (see Supplementary Tab. 4 and 8).

We recognised, that indoor and human samples can be distinguished by their abundance and prevalence of *Thaumarchaeota* and *Euryarchaeota*, with *Thaumarchaeota* being highly enriched in indoor samples ($P_{adj} = 3.5*10^{-11}$; see Supplementary Fig. 5; Mann–Whitney U test), and *Euryarchaeota* being highly enriched in human samples ($P_{adj} = 1.3*10^{-5}$). This especially applies to signatures from *Candidatus Nitrososphaera* ($P_{adj} = 2.9*10^{-26}$) and *Cand. Nitrosocosmicus* ($P_{adj} = 1.1*10^{-24}$) that were rarely abundant in samples from the human body, but frequently occurred in the analysed indoor samples. Also, the relative abundance of unassigned *Nitrososphaeraceae* that were found in skin, urine and nasal samples was significantly increased ($P_{adj} = 1.0*10^{-20}$) in indoor samples.

Nevertheless, several archaeal genera were shared in both indoor samples and human-associated samples. For example, reads classified to *Methanobacterium**, *Methanobrevibacter**, *Methanosarcina**, *Halococcus*, unassigned *Nitrososphaeraceae* and *Woesearchaeia* were present in both, indicating overlap and exchange between both microbiomes.

Venn Diagrams were used to display the number of shared archaeal ASVs between the indoor environments and all analysed body sites. Each sample type contained a certain number of specific ASVs (e.g. nasal samples: 489 ASVs, oral: 41 ASVs, skin: 5 ASVs, stool: 24 ASVs, indoor samples: 267 ASVs). The vaginal samples revealed only little overlap with other samples with respect to archaeal ASVs (15 unique ASVs, two shared with urine samples). Notable, nine ASVs (*Methanobrevibacter**) were shared amongst all sites, and the skin samples shared most (20) ASVs with the indoor environment (four *Candidatus Nitrosocosmicus* ASVs and 16 ASVs uncultured *Nitrososphaeraceae*) (Fig. 4e,f).

In the next step, we focused further on the signatures of methanogenic archaea, as we used them as specific model microorganisms in our study. We were interested, whether the methanogens identified in the indoor samples were of human origin or rather associated with archaeal signatures from the natural environment. For this, we firstly extracted methanoarchaeal sequences from our indoor datasets - PMA and nonPMA, which were then used together with reference sequences available from different environments through official databases to generate a phylogenetic tree (Fig. 5).

Most of the *Methanobrevibacter* sequences identified in the indoor samples clustered together with host-associated (human (*M. smithii**, *M. oralis**), bovine/ovine (*M. millerae**), or biogas reactor/anaerobic digestor-derived sequences, which could probably also be of holobiont origin. Due to the fact that *Methanobrevibacter** is considered as a strongly host-associated taxon (Maria Chibani et al., 2020), we envisage that our detected *Methanobrevibacter** signatures are mostly derived from human and only rarely from environmental environments.

Borrel et al. (2017) have shown that nearly all human-associated *Methanomassiliicoccales** DNA sequences clustered in a host-associated clade together with other *Methanomassiliicoccales** sequences identified in animals, with the exception of two taxa, namely Candidatus *Methanomassiliicoccus intestinalis** and *Methanomassiliicoccus luminyensis**. These two taxa, although they have been identified and isolated from human samples, cluster together with sequences mainly associated with soil and sediment environment ("environmental clade"), clearly showing that some environmental *Methanomassiliicoccales** taxa can be transferred from the environment to the human body and can colonise the human gut. The sequences assigned to the order of *Methanomassiliicoccales** in our samples clustered only in the environment-associated clade and were closer to *Methanomassiliicoccales** sequences identified in soils, sediments and anaerobic digesters. Therefore, the environment appears to be the most probable source of *Methanomassiliicoccales** sequences identified in our bathroom floor samples.

Signatures of *Methanobacterium** are being more and more associated with human origin (Borrel et al., 2020; Maria Chibani et al., 2020). For *Methanobacterium**, we included sequences from environmental- and human-associated taxa in order to determine whether *Methanobacterium** sequences identified on the bathroom floor were of human origin or not. Most *Methanobacterium** sequences identified in our human dataset clustered separately to all other *Methanobacterium** sequences, with the exception of one ASV, which clustered together with *Methanobacterium ferruginis** and ASV49 identified in the nonPMA samples. Seven ASVs from PMA samples clustered with *Methanobacterium oryzae** and shared a node with the phylogenetic group formed by the human-associated ASVs.

Phylogenetic and abundance-based analyses indicate that probably most of the identified methanogenic sequences (particularly *Methanobrevibacter** and *Methanobacterium*)* in the bathroom floor samples are indeed of human-origin, especially for identified sequences in indoor samples.

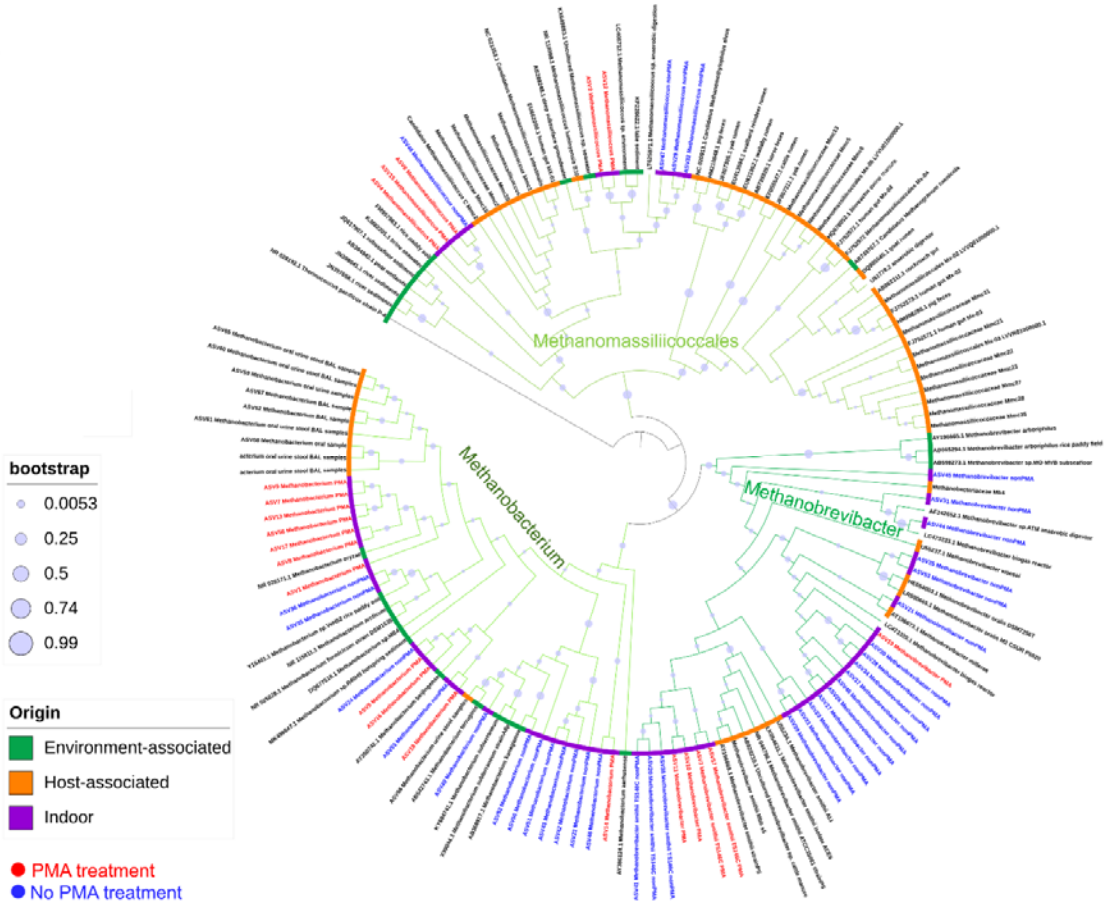


Fig. 5. Phylogenetic tree based on the sequences obtained from our study, NCBI, and two other studies (Borrel et al., 2017; Pasolli et al., 2019)**.** The circle indicates the origin of the sequences used for creating the tree (see legend). The branches of the tree were coloured in different shades of green according to the taxa they represent.

Human-associated methanogens can survive oxygen exposure up to 48 hours

Methanogenic archaea are strict anaerobes and are regarded to be highly oxygen-sensitive. Nevertheless, in households with methanogenic taxa, we frequently observe them in both nonPMA and PMA samples, indicating a certain tolerance towards stressors in the BE. Therefore, we aimed to experimentally analyse whether methanogenic archaea, serving as models for strict anaerobic gastrointestinal microorganisms, were able to survive under

aerial oxygen conditions and could thus, serve as a potential source for colonising the human body.

Three methanogen strains previously isolated from the human body, namely *Methanobrevibacter smithii** (DSM no. 2375), *Methanosphaera stadtmanae** (DSM no. 3091), *Methanomassiliicoccus luminyensis** (DSM no. 25720), and one recently obtained isolate (*Methanobrevibacter sp.**; unpublished) from human faeces, were exposed to aerobic and anoxic conditions over the period of up to 7 days (0h, 6h, 24h, 48h and 168h). After exposure, the cells were transferred to fresh anoxic media and were allowed to grow for 2-3 weeks. Growth was then tested by measuring OD_{600} and methane production. All those four strains were able to survive in the aerobic environment for at least 6h (Fig. 6). *Methanomassiliicoccus luminyensis** was even able to survive for more than 48h of exposure to aerobic conditions. It shall be noted that no growth of *Methanomassiliicoccus luminyensis** was detected after 7d of cultivation, neither under anoxic nor oxic conditions, indicating potential other negative influences (e.g. starvation) hindering normal outgrowth.

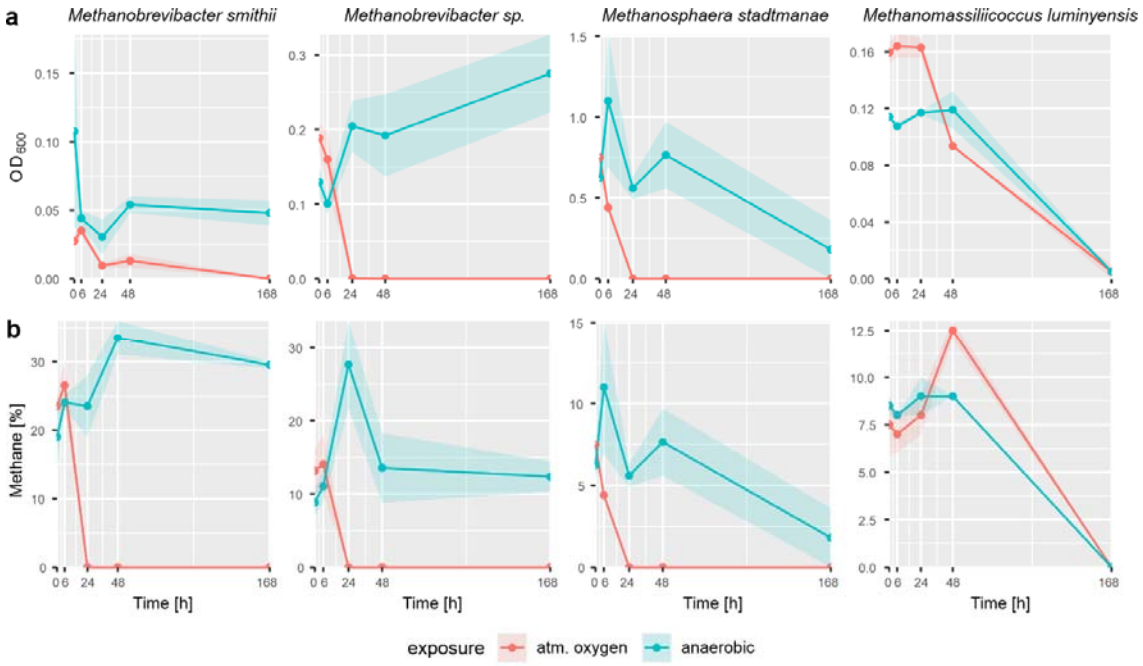


Fig. 6. Observed growth of selected human-associated methanogens after exposure to 21% oxygen (air). Plots indicate the growth as determined by **(a)** OD_{600} measurements and **(b)** methane production of the tested strains after exposure to an aerobic (red) or anaerobic (blue) environment for different durations (n = 2).

In order to retrieve information on the genomic capacity of methanogens to resist oxygenic stress, we analysed available representative genomes of *M. stadtmanae**, *M. smithii**, and *Methanomassiliicoccus** (Tab. 1). We specifically searched for key-stone genes, as identified in Lyu & Lu (2018), and used the Microbial Genome Annotation & Analysis Platform (MaGe) for detailed comparative genomics (Vallenet et al., 2006).

Tab. 1. Genes for O_2/reactive oxygen species elimination enzymes and their presence in genomes of human-associated methanogens (Lyu & Lu, 2018)**. Explanation of letters in brackets: (a)** protein inferred from homology, **(b)** predicted protein, **(c)** putative, **(d)** ferritin-like, **(e)** function from experimental evidence in other organisms, **(f)** ferritin, dps family protein. Specific gene identifiers derived from MaGe microscope database (Vallenet et al., 2006) and UniProtKB (Bateman, 2019) are displayed in each column when present. n.d.: not detected.

		Methanosphaera stadtmanae		*Methanobrevibacter smithii*			*Methanomassiliicoccus*	
Function	**Enzyme**	**DSM 3091**	**DEW79**	**DSM2374**	**DSM861**	**DSM 2375 (ALI)**	**M. luminyensis B10**	**M. intestinalis strain Issoire-Mx1**
$2\ H_2O_2 \rightarrow O_2 + 2\ H_2O$	Catalase (EC 1.11.1.6)	n.d.	n.d.	n.d.	n.d.	n.d.	CAJEv1_180010 (e)	MMINT_12710 (a) MMINT_12690 (a)
$H_2O_2 + 2\ e + 2\ H^+ \rightarrow 2\ H_2O$	Peroxiredoxin (EC 1.11.1.24)	n.d.	n.d.	n.d.	n.d.	n.d.	CAJEv1_50026	MMINT_19160 (a) MMINT_05530 (b) MMINT_15900 (b)
metalloprotein involved in oxidative stress tolerance	Rubrerythrin	Msp_0789 (b)	n.d.	METSMIF1_02444 (b)	Msm_1733 (b) Msm_1348 (b)	METSMIALI_01290 (b)	CAJEv1_240034 (e)	MMINT_05220 (b) MMINT_00570 (b)
$2\ O_2^- + 2\ H^+ \rightarrow H_2O_2 + O_2$	Superoxide dismutase (EC 1.15.1.1)	n.d.	n.d.	n.d.	n.d.	n.d.	CAJEv1_120763 (e)	n.d.
$O_2^- + 2\ H^+ \rightarrow H_2O_2$	Superoxide reductase	n.d.	n.d.	METSMIF1_03626 (a,c)	n.d.	METSMIALI_00110 (a,c)	n.d.	n.d.
$O_2 + 2\ F_{420}H_2 \rightarrow 2\ F_{420} + 2\ H_2O$	$F_{420}H_2$ oxidase	n.d.	n.d.	n.d.	Msm_1349 (b)	n.d.	n.d.	n.d.
$2\ NO + 2\ e + 2\ H^+ \rightarrow N_2O + H_2O$ $O_2 + 4\ e + 4\ H^+ \rightarrow 2\ H_2O$	NO/O_2 reductase	n.d.	n.d.	n.d.	n.d.	n.d.	CAJEv1_120660 (e)	n.d.
Iron storage	Ferritin	Msp_0852 (a, b)	n.d.	METSMIF1_03296 (a,d)	Msm_1712 (a, b)	METSMIALI_00411 (a,d)	CAJEv1_120620 (e, f)	MMINT_08340 (a, f)
Redox buffering, cell redox homeostasis	Thioredoxin	Msp_0103 (a,c) Msp_0820 (a,c)	n.d.	METSMIF1_02203 (a)	Msm_0838 (a, c)	METSMIALI_01538 (a)	CAJEv1_200051 CAJEv1_230019 (c,) CAJEv1_240002	MMINT_17530 (b) MMINT_19530 (b)
Iron ion binding	Rubredoxin (EC 1.4.3)	Msp_0445 (a) Msp_0444 (a)	n.d.	METSMIF1_03551 (a) METSMIF1_03550 (b) METSMIF1_03395 (a) METSMIF1_03318 (b)	Msm_0187 (b) Msm_0188 (a)	METSMIALI_00388 (b) METSMIALI_00192 (b) METSMIALI_00191 (a) METSMIALI_00344 (a)	CAJEv1_120430 (e)	MMINT_08590 (a)
Used database for analysis	UniProtKB	UniProtKB	UniProtKB	UniProtKB	UniProtKB	UniProtKB	MAGE	UniProtKB

All investigated methanogens (except DEW79) revealed the presence of rubrerythrin, ferritin, thioredoxin and rubredoxin in their genomic inventory. The extended survival under

oxygenated conditions for *Methanomassiliicoccus** was supported by the detection of catalase and peroxiredoxin, as well as superoxide dismutase in the case of *M. luminyensis**.

Overall, based on experimental and database-derived information, we can state that human-associated archaea have profound capacities to survive under oxygenated conditions for a minimum of 6 hours.

Discussion

The viability of microorganisms that exist in indoor environments is of great interest (Gilbert & Stephens, 2018), as a substantial amount of microorganisms is in constant exchange with the human body (B. Brooks et al., 2014; Lax et al., 2014; Lax, Smith, et al., 2017; Qian et al., 2012). The interaction with (viable) microbes is arguably of crucial importance for the training of the immune system and sensitisation in infants (Shan et al., 2019), as well as the (re-)colonisation of the human microbiome after antibiotic interventions or disease.

In our study, we could confirm that the indoor surfaces of normal houses harbour unique microbial communities (Lax et al., 2014). The single household profiles are largely driven by human microbial signatures and only to a lesser extent by environmental taxa (for comparison, please see Adams et al., 2015; Chase et al., 2016; Hewitt et al., 2012; Jeon et al., 2013; Rintala et al., 2008), (Fig. 3). This is in agreement with the concept of a personalised microbial cloud mediating the transfer to surfaces (Meadow et al., 2015). In particular, the skin microbiome was identified as a major contributor (~40%), even in areas close to the toilet (Adams et al., 2015; Flores et al., 2011; Gibbons et al., 2015). Even though a substantial variability was observed among the ten households, still approximately 10% of the overall microbial community of bathroom floors was formed by microbiomes from faecal, vaginal and urinary origins. The presence of those microbial taxa is likely to be explained by aerosols that are dispersed by toilet flushing (Prussin & Marr, 2015). However, closing of the toilet lid prior to flushing, as practised in households H4 and H10, did not substantially alter the proportions of contributing body sites and raises the question whether there are other possible sources such as the shower (Feazel et al., 2009) or the sink (Dunn et al., 2013).

Molecular surveys of the BE microbiome usually do not discriminate between viable and dead microbes. As DNA is very stable in the environment, and signatures of microbes remain detectable for a long time, the presence of DNA signatures alone does not serve as a

good indicator for viability and metabolic activity per se. Once dispersed, DNA traces of human gut- and skin-associated taxa can still be tracked on public restroom floors and walls after several weeks (Gibbons et al., 2015). Thus, the proportion of relevant microorganisms in the BE might be overestimated in conventional microbiome approaches. Cultivation-based assays, however, are hindered by their technological limitations and might reflect only a non-representative proportion of living indoor microbiota (Gilbert & Stephens, 2018).

In this study, we analysed specifically the intact floor microbial community and determined the quality and quantity of the microbial ex-host survivors by using PMA treatment, which was rarely applied to BE environment samples so far (Emerson et al., 2017; Mahnert et al., 2015; Moissl-Eichinger et al., 2015). One past specific application was the assessment of living microorganisms in vicinity of spacecraft, in order to understand the risk of contamination for extra-terrestrial research targets (planetary protection; Mahnert et al., 2015; Moissl-Eichinger et al., 2015). Notably, a proportion of 1-45% of the detected microbial community was deemed to be alive under (stringent) cleanroom conditions (Moissl-Eichinger et al., 2015; Vaishampayan et al., 2012).

In our experiment, we found that the majority of detected microbial signatures indeed originate from disintegrated cells or free DNA. To be more specific, we observed a more than 3- and 4-fold decrease in the absolute number of 16S rRNA genes analysed via qPCR for archaea and bacteria upon PMA-treatment of bathroom floor samples, respectively (Fig. 3). However, this also implies that after seven days, less than 25% of the microbial signatures originate from intact and likely living cells that are potentially able to colonise the human body. Those potentially living cells detected in PMA samples could be associated with a rather aerobic lifestyle and increased stress tolerance, that includes aerobic human-associated and environmental, but also spore forming taxa. The predominance of aerotolerant and spore-forming taxa in PMA samples supports the notion that microbes do not proliferate, but persist in the BE as a result of accumulation and dormancy (Gibbons, 2016).

Whether obligately anaerobic human commensals are capable of being transmitted between individuals via the BE is still an open question. Notably, microbial taxa such as Clostridia might not be directly transmitted from mother to child, even though strains of Clostridia are the most abundant bacterial group in the maternal gut (Korpela et al., 2018). It is argued that Clostridia likely rely on transmission via relatives or non-direct transmission between hosts, namely being transmitted via the environment (Korpela & de Vos, 2018). This is supported by our observation of an increased survival rate for spore formers, including Clostridia, that are able to outlive oxygen exposure and potentially the acidic environment of the stomach.

It is conceivable that such a horizontal, indirect transfer is a very rare event in healthy adults as the transmission success is dependent on several factors. This includes transmission routes and dispersal efficiency, the ability to survive “ex-host”, human colonisation resistance, as well as the capability to survive gastric and bile acids for gut commensals (Browne et al., 2017). Those factors are largely unexplored for commensals but it can be assumed that pathogens and commensals use similar mechanisms. For example, transmission routes are well known for many pathogens and primarily include the transfer through direct inhalation of aerosols and dust (e.g. *Bacillus anthracis*, *Mycobacterium tuberculosis*) and transmission through surface contact (e.g. *Clostridium difficile**, *Staphylococcus aureus*, *Enterococcus faecalis*) (Gilbert & Stephens, 2018). The very same mechanisms most likely also applies for human commensals to be spread. However, this has to be evaluated by further studies and could be validated by microbial genomic sequence variation analysis to prove the transfer of commensals between individuals via the BE.

Intriguingly, we also detected archaea as integral components of the BE microbiome. Even though archaea were outnumbered by bacteria about 100-fold, they were found in every PMA-treated household sample. Especially taxa of the *Nitrososphaeraceae* were highly predominant in PMA and nonPMA samples and seem to be rather stable components of the BE. In contrast, it appeared that *Euryarchaeota* are only transient occupants of the indoor

microbiome as many genera were limited to a few households and their signals frequently disappeared in PMA samples.

An exception to this pattern was observed for the taxon *Methanobrevibacter**, which is the most abundant human archaeal genus in the GIT (Borrel et al., 2020). *Methanobrevibacter** signatures were found on all analysed human body sites and in seven out of ten household samples in both PMA and nonPMA samples, which appears contradictory to the general assumptions that methanogens are highly oxygen-sensitive (Fetzer & Conrad, 1993; Jarrell, 1985; Thauer et al., 2010; Yuan et al., 2011). However, although *Methanobacteriales** were found to have little capacity to deal with ROS (Lyu & Lu, 2018), experimental data revealed that methanogens can survive oxygen exposure for several hours to days (Ma & Lu, 2011; Ueki et al., 1997; Yuan et al., 2009) and were even found in typical oxygenated environments (Angel et al., 2011, 2012).

By experiments with representative human GIT methanogens, we were able to confirm that indeed *M. smithii** *species* and *M. stadtmanae** are capable of surviving aeration for more than 6 hours, while *M. luminyensis** is even capable of enduring oxygen exposure for more than 48 hours under our experimental conditions (see Fig. 6). The capability of methanogens to endure aerobic conditions was supported by genomic analysis of common pathways to detoxify ROS. All analysed human-associated methanogens, except *Methanosphaera stadtmanae* strain DEW79, possessed key genes for enzymes such as rubrerythrin, ferritin, thioredoxin and rubredoxin to survive aerobic environments (see Tab. 1). *Methanomassiliicoccus sp.* further exhibits genes encoding for putative peroxidases and catalases, making this taxon especially capable of dealing with ROS, as indicated by our experimental approach.

Both the genomic and experimental approaches support the notion of methanogens to endure aerobic conditions for a limited amount of time. This introduces a new potential transmission pathway, which is especially interesting for infants to acquire microbes aside

from direct seeding during vaginal birth. It is yet unknown whether methanogens such as *Methanobrevibacter** are acquired perinatally and escape detection early in life or if they colonise the GIT via another source such as the BE or dietary products like yoghurt, organic milk and vegetables (Brusa et al., 1998; Taffner, Bergna, et al., 2020; Taffner, Laggner, et al., 2020; van de Pol et al., 2017). The latter scenario is plausible as methanogens co-evolved with animals (Borrel et al., 2017; Youngblut et al., 2020) and thus, show strong adaption to the human GIT.

The study presented here is limited by a small sample size and was thus impaired by the high level of heterogeneity among different households. Also, the study did not yet analyse other surfaces/house areas (such as showers or sinks, door handles, dust etc.) which should be included to retrieve a better overall picture (Adams et al., 2015; Dunn et al., 2013). Due to the limitations of amplicon sequencing, functional information for analyses on microbiome capabilities to deal with environmental stressors was not retrievable. Other techniques, such as shotgun metagenomics that allow for taxonomic and functional analysis are advisable for future, PMA-based BE surveys. In addition, environmental, physical parameters such as humidity, temperature or frequency of ventilation should be tracked as those could explain microbial survival (Adams et al., 2017; Xu et al., 2020).

However, by using PMA treatment in combination with molecular quantitative and qualitative methods, this study successfully assessed the survival of Bacteria and Archaea on indoor surfaces. Based on our results, we can argue that one-fifth of the bathroom surface microbiome is intact or even alive. As even strict anaerobes, such as oxygen-sensitive methanogenic archaea, were found to be intact and alive despite the exposure to the fully aerated environment, we can furthermore argue that those could potentially represent a valid source for human microbiome constituents.

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Supplementary files

Supplementary Figure 1. Sampling area of a representative bathroom floor.

Supplementary Figure 2. Analysis of indoor microbiome samples, displaying differences upon PMA treatment on bacterial phyla level. Depicted are taxa that were characteristic to nonPMA and PMA indoor samples. (A) LEfSe analysis, and (B) boxplots of significant different taxa defined by Wilcoxon signed-rank test: *, $P < 0.05$; **, $P < 0.01$.

Supplementary Figure 3. LEfSe analysis of bacterial taxa (phylum, class, order, family, genus-level) depicting differences of PMA and nonPMA indoor samples.

Supplementary Figure 4. PMA treated indoor samples show a significantly decreased relative abundance for some opportunistic pathogens.

Supplementary Figure 5. Comparison of archaeal signatures found on different human body sites (blue) and in the 10 analysed bathrooms (red) on phylum (top) level and for the 25 most abundant genera (bottom). Only samples without PMA treatment were included. Sign.: *, $p < 0.05$; ****, $p < 0.0001$; Wilcoxon signed-rank test.

Supplementary Table 1. Primer pairs used for archaea and bacteria PCR and qPCR.

Supplementary Table 2. The PCR and qPCR conditions for the primer pairs used.

Supplementary Table 3. ASV table and data overview for indoor microbiomes.

Supplementary Table 4. Summary of additional datasets (human microbiome) used in this study. The study including the saliva samples (bacterial dataset), as well as the study on the nasal cavity (archaeal dataset) have already been published (Koskinen et al., 2018; Wolf et al., 2017).

Supplementary Table 5. ASV table of all indoor and human samples, universal dataset.

Supplementary Table 6. List of shared taxa among different households.

Supplementary Table 7. List of most abundant bacterial genera, families and orders and the respective impact of PMA treatment on their relative abundance, analysed by Wilcoxon signed-rank test.

Supplementary Table 8. ASV table of all indoor samples, archaeal dataset.

Supplementary Table 9. ASV table of all indoor and human samples, archaeal dataset.

References in Supplementary Tables: (Amann et al., 1995; Caporaso et al., 2012; DeLong, 1992; El Fantroussi et al., 1999; Klindworth et al., 2013; Raskin et al., 1994)