Genetic exchange shapes ultra-small Patescibacteria metabolic capacities in the terrestrial subsurface

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ABSTRACT

Bacterial genomes are highly dynamic entities, mostly due to the extent of horizontal gene transfer (HGT) occurring in these organisms. HGT is thought to be the main driver of genetic variation and adaptation to local environment in bacteria. However, little is known about the modalities of HGT within natural microbial communities, especially the implications of genetic exchange for streamlined microorganisms such as Patescibacteria (Candidate Phyla Radiation). We searched for evidence of genetic exchange in 125 Patescibacteria genomes recovered from aquifer environments and detected the presence of hundreds of genomic islands, individually transferred genes and prophage combined, with up to 29% of genome length attributed to HGT. Results show that most individual gene transfer events occurred between Patescibacteria, but donors were also phylogenetically diverse groundwater microorganisms. Using gene donor-recipient information, we identified one potential host (Omnitrophota) of the ultra-small bacteria, and confirmed this by matching relative abundance patterns across 16 groundwater samples. A wide variety of metabolic functions were introduced in Patescibacteria genomes by HGT including transcription, translation and DNA replication, recombination and repair. This study illustrates the evolutionarily dynamic nature of Patescibacteria genomes despite the constraints of streamlining, and that HGT in these organisms is also mediated via viral infection.

INTRODUCTION

The horizontal transfer of genetic material (or horizontal gene transfer; HGT), involving acquisition of exogenous DNA, is thought to be a key factor in the evolution of prokaryotes, especially in bacteria (Ochman et al., 2000). HGT accounts for a significant fraction of gene diversity and genomic fluidity in these organisms, leading to a variety of genome structures and physiological diversity (Soucy et al., 2015). This evolutionary process is important for niche adaptation, and is often driven by mobile genetic elements (MGEs) such as genomic islands (GIs), transposons and phage. Many routes exist for gene transfer: uptake of environmental DNA molecules (transformation), exchange between two cellular organisms via a physical bond (conjugation), or delivery through phage infection (transduction).

Recent metagenomic studies of subsurface ecosystems reveal a large group of unusual bacteria referred to as the Patescibacteria phylum (equating to the Candidate Phyla Radiation; CPR), which has been suggested to comprise up to 26% of all currently known bacterial diversity (Parks et al., 2017; Schulz et al., 2017). The group constitutes taxa consistently harbouring small cell and genome sizes (Brown et al., 2015). Implications of reduced size include limited metabolic functions, such as the lack of known amino acid and nucleotide biosynthesis pathways (Castelle et al., 2018). These observations led to the prediction that most ultra-small Patescibacteria have a symbiotic lifestyle (Brown et al., 2015), and some were confirmed to be episymbionts based on cultivation and microscopy studies (He et al., 2021). Close physical associations with other organisms due to metabolic dependencies, along with the long residence time of groundwater (Griebler & Lueders, 2009), and the importance of the biofilms in aquifers (Flynn et al., 2008), could favour genetic exchange in subsurface ultra-small microbial communities.

Despite proximity to other groundwater bacteria, extensive gene loss in Patescibacteria evolution could result in selective pressures against the acquisition and retention of exogenous DNA that are stronger than in non-streamlined genomes. Another potential barrier to HGT lies in the fact that genome reduction in obligate intracellular symbionts has often been associated with loss of functions involved in DNA recombination and repair (Moran & Bennett, 2014), which could limit integration of foreign DNA. Previous studies have, however, shown not only that a significant fraction of Patescibacteria organisms still possesses genes required for homologous recombination (Castelle et al., 2018), but that they are naturally competent for uptake of eDNA molecules, mediated by DNA intake pumps (via comEC and pili complexes; Castelle et al., 2017, 2018; Nelson & Stegen, 2015).

Investigating the mobility of adaptive genes via HGT could provide important information about the evolutionary strategy of these ultra-small organisms, including aspects governing putative host-symbiont interactions. Implications of HGT include altered interaction modalities with host populations, as described in *Vibrio fischeri*. Adding a single gene to the *V. fischeri* genome is sufficient to alter its host range in squids (Mandel et al., 2009). Additionally, acquisition of MGEs has been shown to constitute evidence of symbiotic relationships between HGT recipient and donor, such as extensive gene transfer from *Wolbachia* endosymbionts to their insect hosts during genome reduction (Dunning Hotopp et al., 2007), and putative acquisition of host-derived ankyrin repeat proteins by bacterial sponge symbionts to inhibit phagocytosis (Nguyen et al., 2014). How potentially adaptive genes aid niche adaptation in ultra-small prokaryotes and fine-tune metabolic interactions with their predicted ‘basibiont’ prokaryotic host cells remains largely unknown.

To understand the role of HGT in the evolution of complex, natural groundwater communities, and particularly streamlined Patescibacteria, we analyzed 396 metagenome-assembled genomes (MAGs), including 132 Patescibacteria genomes. We investigated genomic evidence for, and the frequency of, individual horizontal gene transfer events (defined here as HT genes) in the MAGs, and compared these to acquisitions of larger MGEs such as GIs and prophage. Results provide evidence for many HGT events among Patescibacteria, and exchanges with other prokaryotes in groundwater. We report that metabolic functions horizontally acquired by Patescibacteria taxa appear to be biased towards features distinct from the general groundwater communities.

MATERIALS AND METHODS

Sample collection

Groundwater samples were collected from 8 wells from 2 sand/gravel aquifers in Canterbury, New Zealand, as described by (Mosley et al., 2022). Briefly, wells were purged (×3–5 bore volumes), then 3–90 L of groundwater was filtered per sample. Biomass was captured on 0.22 µm mixed cellulose ester (MCE) filters, after passing through a 1.2 µm MCE pre-filter, using a 142 mm filter holder (Merck Millipore Ltd., Cork, Ireland). Samples were preserved in RNAlater (ThermoFisher Scientific, Waltham, MA, USA), transported on dry ice, and stored at -80°C.

After collecting a groundwater sample (as above), a second sample per well was collected following low frequency sonication (2.43 kW for 2 minutes) to induce biofilm (and sediment) detachment from the surrounding aquifer, using a custom sonication device (Close et al., 2020). 0.5–15 L of sediment (and hence biomass) enriched groundwater was filtered as above.

Nucleic acid extraction

To remove RNAlater, filters were centrifuged (2500 g for 5 min) and washed with nuclease-free phosphate-buffered saline (pH 7.4, ThermoFisher Scientific, Waltham, MA, USA). DNA was extracted using the DNeasy PowerSoil Pro Kit (Qiagen, Valencia, CA, USA) and 0.25-0.9 g of whole filter per reaction. Samples were extracted in replicate (1–47 reactions/sample). Replicates were pooled and concentrated using a sodium acetate/ethanol precipitation with glycogen (0.1 µg/µL final concentration, Roche, Basel, Switzerland). High molecular weight DNA was confirmed via gel electrophoresis. DNA was quantified using Qubit fluorometry (ThermoFisher Scientific), and quality was checked using a NanoPhotometer (Implen, Munich, Germany). Extractions yielded 70–864 ng of DNA (8.7 ng for gwj02) for metagenomics.

Metagenome sequencing

Whole genome shotgun sequencing was undertaken on the 16 Canterbury samples (gwj01-16). DNA libraries were prepared using the Illumina TruSeq Nano DNA Kit with ~550 bp inserts, by Otago Genomics (University of Otago, Dunedin, NZ). The low-yield gwj02 sample was prepared with the ThruPLEX DNA-Seq Kit (Takara Bio USA, Inc., Mountain View, CA, USA). Sequences (2×250 bp) were generated using the Illumina HiSeq 2500 V4 platform. Adapter sequences were removed using Cutadapt v2.10 (Martin, 2011), and reads were quality trimmed using sickle v01.33 (parameters -q 30 -l 80). Read quality was checked using FastQC v0.11.7 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/).

Metagenome assembly and binning

The 16 metagenomes were individually assembled using SPAdes v3.11.1 (Bankevich et al., 2012) (parameters: --meta, -k 43,55,77,99,121). Metagenomes from the same well also were co-assembled (same parameters). A dereplicated set of metagenome-assembled genomes (MAGs) (99% average nucleotide identity, ANI, dereplication threshold) was generated as described previously (Tee et al., 2020), and refined according to bin_detangling script (https://github.com/dwwaite/bin_detangling).

For ultra-small prokaryotes, genome completeness and contamination were assessed based on the presence of 51 bacterial single-copy genes (SCGs) for Dependentiae, 43 SCGs for Patescibacteria and 38 archaeal SCGs for DPANN (Anantharaman et al., 2016). For other prokaryotes, completeness was estimated using CheckM v1.0.13 (Parks et al., 2015). To determine coverage, reads were mapped onto the de-replicated MAGs using Bowtie (Langmead et al., 2009). Sample-specific genome relative abundance was calculated by normalizing based on the highest read count between samples (Probst et al., 2018).

Gene prediction and functional annotation

Protein coding sequences were predicted using Prodigal v2.6.3 (Hyatt et al., 2010). Genes were annotated using USEARCH (Edgar, 2010) against UniRef100 (Suzek et al., 2015), Uniprot (The UniProt Consortium, 2017) and KEGG (Kanehisa et al., 2016) databases (e-value ≤0.001 and identity ≥50%), and HMMs against PFAM (Sonnhammer et al., 1998) and TIGRFAM (Haft et al., 2003) databases using

HMMER v3.1b2 (Potter et al., 2018) (e-value ≤0.001). Additionally, functional annotation was carried out using eggNOG-mapper v2 (Huerta-Cepas et al., 2017) against the eggNOG v5.0 database (Huerta-Cepas et al., 2019), using default parameters.

Genome taxonomy

Taxonomic classification of MAGs was performed using the Genome Taxonomy Database Toolkit (GTDB-Tk, v0.2.1) (Chaumeil et al., 2020) with database release 89 (Parks et al., 2020). Maximum likelihood trees were constructed using 120 bacterial and 122 archaeal concatenated marker genes with IQ-TREE v1.6.12 (L.-T. Nguyen et al., 2015) using 100 bootstraps, and ModelFinder (Kalyaanamoorthy et al., 2017) best-fit model LG+F+G4 for bacteria and LG+F+I+G4 for archaea. Trees were visualized and annotated with iTOL (Letunic & Bork, 2007). MAGs (≥80% complete) were compared to GTDB representative genomes (downloaded 20-Jan-2020) by calculating Average Amino-acid Identities (AAI) for blastp matches sharing ≥30% identity over ≥70% of alignment length.

Identification of horizontal gene transfer

Individual HT events among MAGs were identified using MetaCHIP v1.10.0 (Song et al., 2019) at phylum, class, order, family and genus levels. False-positive HT genes (potentially introduced via assembly contamination) were filtered out by removing HT genes marked by MetaCHIP as “full-length match” (where the match represents a large proportion of contig) or “end match” (HT genes located at the end of contig) as described by Dong et al. (2021). Information about HGT events involving members

of the Patescibacteria phylum were retrieved and visualised using the Circlize package in R (Gu et al., 2014).

For genomic island prediction, contigs were concatenated into contiguous sequences and uploaded to IslandViewer4 web server (Bertelli et al., 2017) for genomic island prediction. GIs that were predicted by at least one method, and that did not overlap two concatenated contigs, were considered for the analysis. Genes located on GIs were compared to community gene content using blastn.

Prophage integrated in the Patescibacteria genomes were identified using VIBRANT 1.2.1 (Kieft et al., 2020). Whenever a predicted viral sequence was overlapping a predicted GI, CheckV quality assessment information was used and only “High-quality” genomes were retained as prophage (Nayfach et al., 2021).

Phylogenetic distance

Average Amino-acid Identities (AAI) between MAGs (≥80% complete) involved in HGT events were determined via blastp matches sharing ≥30% identity over ≥50% of query and database alignment length.

Orthology analysis

To investigate the Patescibacteria pangenome, we performed an orthologous clustering of 118,635 protein-coding sequences encoded by the recovered MAGs. Pairwise all-vs-all blastp searches were conducted using the following cut-offs: ≥30% nucleotide identity over ≥50% of query sequence length, e-value ≤0.001. BLAST table outputs were used as input for orthogroup clustering using Orthofinder v2.3.1 (Emms & Kelly, 2019) with -b option and default parameters.

RESULTS AND DISCUSSION

Prevalence of HGT events in Patescibacteria genomes

Using the community-level HGT detection tool MetaCHIP (Song et al., 2019), we investigated the potential horizontal transfer of individual genes between aquifer microorganisms. A total of 1,487 transfers were identified among the 396 groundwater MAGs (Table S1), including 1,407 unique HT genes. Taxonomic breakdown of detected HT events revealed that over half of the Patescibacteria genomes experienced HT with another genome in the dataset, regardless of their taxonomy or estimated genome size (Fig. 1a,b). One hundred and twenty-three (9% of) horizontally acquired genes were identified in 68 Patescibacteria organisms, representing 1.0 ± 1.2 HT (average ± standard deviation) genes received per genome and 1.1 ± 1.3 HT genes per Mbp (Fig. 1b). In comparison, genomes of other members of the community contained on average 3.5 ± 4.8 horizontally transferred genes per genome, but a comparable 1.4 ± 2.1 per Mbp. The comparison of HT events per Mbp indicates that the rate of exchange of individual genes is mostly independent of genome size (i.e. larger genomes do not receive proportionally more genes). This is supported by significant positive correlations between HT events per genome and estimated genome size (both donor and recipient HT events, Fig. 2), and is consistent with previous observations of HT acquired genes in prokaryotic genomes (Garcia-Vallvé et al., 2000), and of transfers occurring between distantly related organisms (i.e. cross-order transfers, >85% of the total HT events detected here) (Cordero & Hogeweg, 2009).

When searching for large MGEs, we found 120 GIs in 63 Patescibacteria MAGs, ranging from 2.7 to 45 kbp long, with 40 GIs over 10 kb long (average length 17.4 ± 0.7 kb) (Table S2). Additionally, 24 putative prophage genomes (30% of those

overall) were found to be concentrated in 5 Patescibacteria MAGs – with the proportion of prophage in Patescibacteria being comparable to the proportion of Patescibacteria MAGs in the community (33%). This frequency of prophage in ultra-small bacterial genomes is surprising, as prophage frequency has been shown to increase with genome size (Touchon et al., 2016). Accordingly, our analysis shows that, as also observed for HT genes, the frequency of acquisition of longer complex DNA sequences, constituting GIs, increased with the genome size of groundwater-derived MAGs (Fig. 2b).

When considering the groundwater community overall, the proportion of genome length occupied by GIs and prophages, combined, remained constant regardless of recipient genome size (Fig. 2b), and despite GI and prophage sequences being similar in length between ultra-small and other microorganisms (Fig. 3, Table S2). This is consistent with observations, by Ochman et al. (2000), that the proportion of horizontally transferred genes is independent of the amount of protein-coding sequences in complete prokaryotic genomes. However, when comparing the two community fractions, results here show the proportion of genome length occupied by putative GIs and prophage was slightly, but significantly higher in Patescibacteria than the rest of the communities – on average 3.0 ± 4.4% versus 2.0 ± 2.8%, respectively (Fig. 1c).

The extent of gene transfer events detected here is comparable to those detected in a variety of other phylogenetically diverse microbial communities, e.g. 90.4% of groundwater MAGs containing detectable HGT events, compared to 78.8% in cheese-associated communities, and 89.5% in prokaryotic isolate genomes (Bonham et al., 2017; Ochman et al., 2000). However, the extent of genetic exchange observed in the groundwater microbial community is likely

underestimated. Ancient transfer events will escape detection due to robust integration within the recipient genome via modification of GC content and tetranucleotide frequencies (Lawrence & Ochman, 1997). Moreover, while MetaCHIP has proven efficient in predicting HGT donors/recipients relationships within microbial communities, donor taxa might not have been captured in the MAG dataset.

HGT origins in Patescibacteria taxa

Most HT events detected occurred between members of the Patescibacteria (Fig. 4a), with 104 of the 123 HT genes received by Patescibacteria originating from counterparts. This observation is consistent with research, which demonstrates that HGT is most prevalent among closely related organisms (Bonham et al., 2017), and that intra-species HGT occurs at far greater frequency (Majewski et al., 2000). HGT was likely also facilitated by the spatial proximity of microorganisms (Gogarten et al., 2002), in particular by microbial cohorts in groundwater communities (Hug et al., 2015). This includes cohorts formed by ultra-small prokaryotes, where individuals spatially co-occur as described previously in subsurface and soil ecosystems (Geesink et al., 2020; Herrmann et al., 2019; Nicolas et al., 2021).

Although a smaller number of MAGs were recovered from Omnitrophota and Bacteroidota, organisms in these phyla shared genes more frequently with Patescibacteria (3.5x and 3x more, respectively) than, for example, Proteobacteria, which was the second most populous phylum (Tables 1, S3). Frequent sharing suggests potential close relationships between these organisms and ultra-small prokaryotes (i.e. host/symbiont). The former two bacterial groups have been identified previously as putative HGT donors to Patescibacteria in seafloor

environments (Dong et al., 2021). Moreover, Omnitrophota and Bacteroidota organisms were found to consistently co-occur with the ultra-small bacteria in aquifers (Chaudhari et al., 2021).

We observed further evidence for recent putative horizontal transfers to groundwater Patescibacteria from patescibacterial counterparts and other taxa based on positive correlations between the relative abundances of HGT pairs across sites (29 out of 131 pairs involving Patescibacteria as donor or recipient with Pearson's correlation coefficient >0.5, $p < 0.05$, Table S4). For example, Patescibacteria nzgw387 received a copy of the *lysR* gene from Omnitrophota nzgw326 (Fig. 4b), a highly conserved transcriptional regulator involved in numerous cellular functions (Maddocks & Oyston, 2008), and is shown to co-occur with its donor, Omnitrophota nzgw326, in groundwater (Fig. 4c).

A BLAST analysis of GI genes against the gene content of whole groundwater communities showed that most of the genes located on GIs could not be matched to any organisms within the communities (91.9% of GI genes; Table S5). Nonetheless, we found that 7% of GI genes were related to those in Patescibacteria other than the one carrying the GI. Interestingly, the content of one 4,250 bp long GI in Patescibacteria nzgw468 (74% genome completeness, no contamination) was highly similar to genes in the archaeon Halobacterota nzgw24 (99% completeness, 1.3% contaminated), indicating a potential recent transfer event between the two distantly related organisms. None of the 11 genes located on the GI could be functionally characterized (as for a large proportion of genes in Patescibacteria genomes), but were between 85 and 100% identical in amino acid composition to the ones of nzgw24. While inter-domain transfers do occur between Archaea and Bacteria, they are less frequent than bacterial-domain confined HGT events (Nelson et al., 1999,

López-García et al., 2015). Gene exchange between Patescibacteria and their archaeal ultra-small counterparts (DPANN) has been reported (León-Zayas et al., 2017), particularly in the context of membrane-associated protein encoding genes potentially involved in host-symbiont interactions (Jaffe et al., 2016). Accordingly, we detected 9 HT events from Patescibacteria to DPANN archaea (Micrarchaeota and Nanoarchaeota; function of transferred genes discussed below). A further, 2 genes (one ABC-transporter and one gene related to the BRO family, of which the function remains unknown) were transferred from Nanoarchaeota to Patescibacteria (Table S6).

Metabolic functions encoded in horizontally acquired regions

Patescibacteria, or CPR bacteria, have reduced genomes and limited biosynthetic capacities (Castelle et al., 2018). The metabolic function of horizontally transferred genes that are retained in Patescibacteria genomes could provide clues about the evolution of dependencies in these ultra-small prokaryotes regarding their symbiotic interactions with hosts. Although a substantial portion of genes located in horizontally acquired genomic regions could not be assigned any function (61.6% of all genes for Patescibacteria, 38.9% for other taxa), genes located in those regions in Patescibacteria genomes that were able to be annotated are involved in a wide range of metabolic pathways (Fig. 5), and include a large number of genes involved in translation and transcription. Horizontally acquired translation and transcription genes predominantly comprised ribosomal proteins (small and large subunits), elongation factor *tuf*, which plays a central role in protein synthesis, and chaperonin *groS* (Table S6). These results reflect trends identified when considering the most frequently transferred genes to other groundwater prokaryotes, which notably

included the *rpsL* gene encoding ribosomal protein S12 and transcription initiation factor *infA*, alongside translation elongation factor *tuf*. Kanhere and colleagues described similar findings (Kanhere & Vingron, 2009). By inferring HGT events based on evolutionary distances within orthologous protein families (using the COG database), authors found that genes exchanged among bacteria were primarily involved in translation (Kanhere & Vingron, 2009).

HT genes in Patescibacteria were found to be highly enriched, compared to other phyla, in genes assigned to the EggNOG “Defense mechanisms” category (Fig. 5). These included genes (e.g. *macB* and *msbA*) encoding ABC-type systems for multidrug and antimicrobial peptide transport. Membrane-located multidrug transporters have been identified previously in relatives of ultra-small bacterium *Babela massiliensis* (Dependentiae phylum, previously Candidate division TM6). Their specialization is thought to be important at the host-symbiont interface (Jaffe et al., 2016). There is growing evidence that drug transporters are required for survival of intracellular pathogenic bacteria in host cells (Du et al., 2015). Genes assigned to chromatin structure were also more prevalent in the HT gene pool in Patescibacteria. Among these genes were six SWIB/MDM2 domain containing proteins, which are chromatin-remodelling proteins involved in transcriptional activation (Tang et al., 2010), and were almost exclusively transferred between Patescibacteria (Table S6). DNA packaging in the form of chromatin is well documented in Eukaryotes and Archaea, but remains poorly understood in Bacteria (Shen & Landick, 2019). Whether ultra-small bacteria use chromatin-remodelling proteins to manipulate their own genomic DNA structure, or for the modification of eukaryotic/archaeal symbiotic host DNA is unclear.

In contrast, other groundwater taxa more frequently acquired genes involved in energy production (respiratory chain) and amino acid biosynthesis, which Patescibacteria are known to consistently lack (Castelle et al., 2018). This could further indicate that ultra-small bacteria must scavenge resources such as protons and amino acids from the host or surrounding environment. A higher proportion of genes involved in secondary metabolite pathways and signal transduction mechanisms was also detected in other taxa (426 HT and GI genes in 105 'other' MAGs versus 18 genes in 15 ultra-small MAGs) (Table S6). Secondary metabolites are considered luxury metabolism (Demain & Fang, 2000) and can constitute public goods (e.g. siderophores) (Weigert & Kümmerli, 2017). As such, biosynthetic gene clusters encoding for these metabolites are uncommon in streamlined genomes (Konstantinidis & Tiedje, 2004; Sharrar et al., 2020), which have reduced auxiliary functions (Giovannoni et al., 2014), and are more likely to depend on the metabolites produced by other organisms (Morris et al., 2012). Similarly, loss of signal transduction mechanisms, which facilitate environmental monitoring, is a characteristic feature of streamlined bacterial genomes, including those of bacteria with non-intracellular lifestyles (e.g. bacterioplankton) (Gabaldón & Huynen, 2007; Swan et al., 2013).

Although it has been shown that gene function impacts HGT overall, studies assessing functional categories of horizontally transferred genes have reported diverging results. The 'complexity hypothesis' suggests that genes involved in cellular housekeeping are more frequently subjected to horizontal transfer, while informational genes (involved in transcription, translation, and related processes are more resistant to transfers (Jain et al., 1999). Nakamura et al. (2004) found that horizontally transferred genes are biased towards cell surface, DNA binding and

pathogenicity related functions. Conversely, other studies have found that the functional category of a gene family is an insignificant factor in determining HGT (Choi & Kim, 2007; Cohen et al., 2011). While we found that the genes localized in GIs harbour similar metabolic functions overall between Patescibacteria and the rest of the groundwater community, functions of individual genes acquired by the ultra-small bacteria through horizontal transfer tend to differ (Fig. 5). Results obtained here therefore suggest lifestyle of the recipient impacts the traits of genes transferred and also retained.

Biases towards horizontally acquired features distinct from the general groundwater communities by Patescibacteria may be explained by constraints on streamlined genomes. Selection of distinct features could occur by two mechanisms: (i) retention of horizontally transferred genes and concentration due to selective deletion of other less desirable features, and (ii) selective retention of acquired genes with desirable features. The caveat being that the distribution of functions among the hypothetical coding DNA sequence (CDS) fraction in Patescibacteria is unknown.

We could question whether the length of transferred DNA is an important factor affecting integration and retainment of transferred genes and related metabolic functions in streamlined ultra-small and other groundwater prokaryotes. The length of transferred DNA was 10,324 ± 9,397 bp on average for the 1,046 detected GIs (10.8 Mbp total in all groundwater taxa) and 899 ± 562 bp for the 1,501 HT genes (1.3 Mbp overall) (Table S2). Therefore, while a third less GIs than individually transferred genes were detected in terms of number, the total length of sequences transferred was substantially greater with GIs, and broadly equivalent between Patescibacteria (9,689 bp per MAG) and other groundwater microorganisms (10,406

bp per MAG). Besides sequence length, it has been shown that recombination efficiency decreases exponentially with sequence divergence (Majewski et al., 2000; Majewski & Cohan, 1999). However, the presence of flanking regions of identity in exchangeable regions can remove most barriers to recombination. The shortest length of sequence homology necessary for efficient recombination can vary greatly depending on the organism, the recombination pathway used, and other factors (Thomas & Nielsen, 2005). In *Escherichia coli*, efficient recombination has been observed with as little as 23 bp of sequence homology (Shen & Huang, 1986).

Impact of phylogenetic distance and direction of transfer

We assessed how phylogenetic distance between donor-recipient pairs associates with the metabolic function of genes acquired by Patescibacteria. Genetic exchange occurred over a wide range of phylogenetic distances based on average amino acid identities between genomes of groundwater microorganisms (as low as 38.5% AAI, Fig. 6a). Within the Patescibacteria phylum, genes of diverse metabolic functions were transferred regardless of phylogenetic distance (41.0-54.3% AAI). However, direction of HT events (between members of the Patescibacteria phylum, other groundwater microorganisms to Patescibacteria, and Patescibacteria to other groundwater microorganisms) appeared to have an impact on the metabolic function of acquired genes (Fig. 6b). A number of metabolic functions were found to be exclusively transferred between Patescibacteria (encoded by 12 out of 104 HT genes shared between Patescibacteria), and were involved in processes such as amino acid metabolism (serine hydroxymethyltransferase, GlyA), cell division (actin homolog, MreB), intracellular trafficking, co-enzyme metabolism (riboflavin biosynthesis) and inorganic ion metabolism (cation transport) (Fig. 6b, Table S6).

Others were exclusively shared from Patescibacteria to other phyla (out of n=29 transfers overall, Fig. 6b), namely two genes associated with chromatin structure and dynamics (SWIB/MDM2 domain-containing protein) and carbohydrate metabolism (mannose-6-phosphate isomerase, COG0662). Moreover, Patescibacteria donated a greater fraction of replication, recombination and repair genes (GIY-YIG catalytic domain-containing protein, UvrD subfamily helicase, bacterial nucleoid DNA-binding protein COG0776, DNA-3-methyladenine glycosylase) to other bacteria. Among the nine HT events from Patescibacteria to DPANN archaea reported above, three were related to a glyoxalase (Table S6), which is key metalloenzyme in the glycolytic pathway involved in the detoxification of reactive methylglyoxal into D-lactate. It is a common feature across domains of life, including in Archaea (Rawat & Maupin-Furlow, 2020), and was previously reported in CPR single amplified genomes (León-Zayas et al., 2017). The reason ultra-small archaea exhibit an apparent preference for acquiring bacterial homologs is unclear.

A very small number of HGT events (two) also occurred exclusively from other prokaryotes to Patescibacteria (out of n=20 transfers overall, Fig 6b). This includes the *acpP* gene encoding an acyl carrier protein, and a gene encoding a putative lysophospholipase (COG2267, alpha beta hydrolase superfamily). Lysophospholipases catalyse phospholipid hydrolysis and generate fatty acids. A recent study coupling lipidomics and metagenomics approaches suggested that members of the Patescibacteria phylum, that lack capacities for fatty acid biosynthesis (Castelle et al., 2018), are able to recycle lipids from other bacteria for cell membrane biogenesis (Probst et al., 2020). This suggests that HGT could facilitate the sourcing of fatty acids in Patescibacteria. Phospholipase genes have also been reported in the genomes of other ultra-small CPR prokaryotes (for

example, in amoeba endosymbiont *Vampirococcus lugosii*; Moreira et al., 2021). Patescibacteria were also more likely to receive genes from other groundwater microorganisms associated with nucleotide metabolism (n=2), including one thymidylate synthase genes *thyA.* The enzyme encoded catalyzes the *de novo* synthesis of dTMP (deoxythymidine monophosphate), an essential precursor for DNA biosynthesis (Voeller et al., 1995), which is a capacity already encoded by the minimal biosynthetic gene repertoire of groundwater Patescibacteria. Our results indicate that this essential function can also be horizontally acquired from other members of groundwater microbial communities.

While the number of transfer events recorded from general groundwater microorganisms to Patescibacteria and vice versa is low, limiting robust conclusions, these numbers are consistent with known associations between HGT and phylogenetic distance among prokaryotes more generally (Majewski et al., 2000). Overall, metabolic functions gained via HGT in ultra-small prokaryotes remain poorly studied, regardless of phylogenetic origin. Events identified here tend to confer Patescibacteria with critical functions, such as the ability to degrade lipids, and carry out the biosynthesis of nucleotides.

Patescibacteria pangenome and HGT

The evolutionary fate of a HGT event is determined by fitness conferred to the recipient genome and cell, i.e., whether the acquisition of exogenous DNA is beneficial, neutral or deleterious. In order to evaluate the importance of the metabolic functions acquired through HGT in Patescibacteria, we performed a pangenome analysis of the 125 reconstructed MAGs. The pangenome of all the examined Patescibacteria contained 7,732 orthogroups, comprising 88,187 genes (74.3%) out

of the total 118,635 predicted genes. Genes in the pan-genome were classified into 3 groups based on their occurrence: (1) the “core” genes, which are present in at least 2/3 of all 132 genomes, (2) the “shared” genes are found in more than one genome, but are not core genes, and (3) the “unique” genes, comprising species-specific genes (i.e. genes present in one genome only) and genes not assigned to an orthogroup. Both shared and unique genes comprise the accessory genome. Results indicated the vast majority of orthogroups (96%) were shared between the groundwater Patescibacteria, and only 3% of orthogroups (2,634 genes) comprised the core genome (Fig. 6c). This represents the lower end of core genome size among bacterial pangenomes (Bosi et al., 2017; Shin et al., 2016) and could be partially due to MAG incompleteness, but is consistent with the variation found in the core protein families by Méheust and colleagues across members of the CPR radiation (Méheust et al., 2019).

GIs brought many unique, and hence divergent, genes to the Patescibacteria pangenome (41.2% of GI genes, Fig. 6c). These observations are consistent with previous work suggesting that prokaryotes contain a higher proportion of novel genes in GIs compared with the rest of their genome (Hsiao et al., 2005). HT genes acquired by Patescibacteria species were spread across the core and the shared gene fractions of these organisms (45.8% shared and 51.9% core, Fig. 6c), and not the unique fraction. A high proportion of core or shared genes would be expected given predicted transfers occurred mostly between Patescibacteria species (83.2% of HT events, Fig. 4a). However, the lack of any unique genes reflects limitations in the detection of HT events by the MetaCHIP tool, which considers self matches to be false positive (i.e. a donor is required) (Song et al., 2019).

Streamlined genomes are characterised by low numbers of paralogs (Giovannoni et al., 2014). Accordingly, among all genes in Patescibacteria, we detected a low number of paralogous orthogroups based on the pangenome analysis results (89 genes in 36 species-specific orthogroups – i.e. 89 paralogs out of 118,635 genes). We found that no genes located on GI regions nor HT genes were paralogs of other genes in the Patescibacteria pangenome. Gene duplicates are generally under purifying selection (Lynch & Conery, 2000), and have been shown to evolve faster than non-duplicated genes with a similar level of divergence (Kondrashov et al., 2002), introducing new proteins. Nonetheless, gene duplication is usually widespread in bacterial genomes, with an estimated 7–41% of bacterial proteins encoded by paralogs (Gevers et al., 2004), and is a common strategy for creating novel gene functions for niche expansion in prokaryotes (Hooper & Berg, 2003). However, it has been quantitatively demonstrated that gene gain via HGT rather than gene duplication is the principal factor of innovation in the evolution of prokaryotes (Treangen & Rocha, 2011). Based on a comparably high proportion of HGT into Patescibacteria as for other phyla, results here demonstrate HGT, and unique genes conferred by GIs, are almost exclusively used over duplication by Patescibacteria to expand niche ranges.

GI in Patescibacteria nzgw456 encoding a full type I restriction-modification system

Streamlined bacteria (and archaea) have been hypothesized to be largely lacking classical defense mechanisms, such as CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats – CRISPR associated), restriction-modification (R-M) and toxin-antitoxin systems (Koonin et al., 2017). Similarly, the

analysis of hundreds of CPR/Patescibacteria genomes recovered from diverse environments revealed that most are missing CRISPR-Cas adaptive immune systems, lost in the process of genome streamlining, and instead rely nonspecific defence, such as R-M systems (Castelle et al., 2018). Although first believed to constitute a barrier to genetic exchange between organisms (Vasu & Nagaraja, 2013), it has been recently suggested that these systems play an important role in HGT rates by increasing the frequency of exchange in organisms possessing numerous R-M systems (Oliveira et al., 2016).

Out of the 120 GIs detected in Patescibacteria we identified four encoding restriction modification systems or endonucleases in patescibacterial genomes. All four MAGs contained a high number of GIs compared to other patescibacterial counterparts (average 5 ± 2.8 SD vs 1.7 ± 1.0), and three acquired more HT per Mbp than the average for Patescibacteria overall (average 2.5 ± 1.3 SD vs 1.2 ± 1.5, Table S2). One GI in Patescibacteria nzgw456 (Paceibacteria class) encodes a full type I R-M system (Fig. 7). Type I R-M systems, which are the most complex of all four known R-M systems (Murray, 2000), comprise three subunits, namely HsdR and HsdM (required for methyltransferase activity), and HsdS (required for restriction). Co-located with genes encoding these subunits on the GI was one putative ATP-dependent endonuclease. Type I R-M related genes were also found in a GI present in Patescibacteria nzgw350, and other restriction endonucleases in nzgw410 and nzgw504 (Table S7). Acquisition of type I R-M genes via HGT was recently described in CPR bacterium *V. lugosii* (Moreira et al., 2021), however not as syntenic genes. It has been suggested that R-M systems could be used by putative epibionts as a way to source of nucleotides by degrading exogenous DNA, including

that derived from viruses (Burstein et al., 2016), in addition to protecting against phage infection (Stern & Sorek, 2011).

CONCLUSION

Recent phylogenetic analyses have shed light on the massive bacterial group that is the Patescibacteria phylum/CPR (Hug et al., 2016). Due to the reductive evolution of Patescibacteria, it is essential to consider the processes of gene gain and loss in respect of the predicted symbiotic lifestyles of these ultra-small microorganisms. Results here indicate that Patescibacteria are involved in dynamic genetic exchange with their ultra-small counterparts and other members of the microbial community. We found a total 124 HT events involving Patescibacteria, 24 prophage and over 100 GIs in Patescibacteria genomes. While most transfers occurred among Patescibacteria, HGT origins were diverse and widespread across both bacterial and archaeal domains, in particular with members of the Omnitrophota and Bacteroidota phyla. A putative host symbiont pair was identified based on HGT evidence and co-occurrence across groundwater samples. Functions encoded by horizontally acquired regions comprised diverse pathways, but mainly transcription, translation and DNA replication, recombination and repair, such as ribosomal proteins and transcription factors. Findings show that acquired genes were distributed across core and accessory portions of Patescibacteria genomes, and that GIs generally introduced phylogenetically distant sequences. Some of the metabolic functions horizontally acquired by Patescibacteria are distinct from those acquired by the general groundwater communities, suggesting unique pressures on gene gain/loss dynamics occurring in ultra-small prokaryotes.

REFERENCES

Anantharaman, K., Brown, C. T., Hug, L. A., Sharon, I., Castelle, C. J., Probst, A. J., Thomas, B. C., Singh, A., Wilkins, M. J., Karaoz, U., Brodie, E. L., Williams, K. H., Hubbard, S. S., & Banfield, J. F. (2016). Thousands of microbial genomes shed light on interconnected biogeochemical processes in an aquifer system. *Nature Communications*, *7*, 1–11. https://doi.org/10.1038/ncomms13219

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., Lesin, V. M., Nikolenko, S. I., Pham, S., Prjibelski, A. D., Pyshkin, A. V, Sirotkin, A. V, Vyahhi, N., Tesler, G., Alekseyev, M. A., & Pevzner, P. A. (2012). SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology□: A Journal of Computational Molecular Cell Biology*, *19*(5), 455–477. https://doi.org/10.1089/cmb.2012.0021

Bertelli, C., Laird, M. R., Williams, K. P., Lau, B. Y., Hoad, G., Winsor, G. L., & Brinkman, F. S. L. (2017). IslandViewer 4: Expanded prediction of genomic islands for larger-scale datasets. *Nucleic Acids Research*, *45*(W1). https://doi.org/10.1093/nar/gkx343

Bonham, K. S., Wolfe, B. E., & Dutton, R. J. (2017). Extensive horizontal gene transfer in cheese-associated bacteria. *ELife*, *6*. https://doi.org/10.7554/eLife.22144

Bosi, E., Fondi, M., Orlandini, V., Perrin, E., Maida, I., de Pascale, D., Tutino, M. L., Parrilli, E., Lo Giudice, A., Filloux, A., & Fani, R. (2017). The pangenome of (Antarctic) Pseudoalteromonas bacteria: Evolutionary and functional insights. *BMC Genomics*, *18*(1). https://doi.org/10.1186/s12864-016-3382-y

Brown, C. T., Hug, L. A., Thomas, B. C., Sharon, I., Castelle, C. J., Singh, A., Wilkins, M. J., Wrighton, K. C., Williams, K. H., & Banfield, J. F. (2015). Unusual

biology across a group comprising more than 15% of domain Bacteria. *Nature*, *523*(7559), 208–211. https://doi.org/10.1038/nature14486

Burstein, D., Sun, C. L., Brown, C. T., Sharon, I., Anantharaman, K., Probst, A. J., Thomas, B. C., & Banfield, J. F. (2016). Major bacterial lineages are essentially devoid of CRISPR-Cas viral defence systems. *Nature Communications*, *7*. https://doi.org/10.1038/ncomms10613

Castelle, C. J., Brown, C. T., Anantharaman, K., Probst, A. J., Huang, R. H., & Banfield, J. F. (2018). Biosynthetic capacity, metabolic variety and unusual biology in the CPR and DPANN radiations. *Nature Reviews Microbiology*, *16*(10), 629–645. https://doi.org/10.1038/s41579-018-0076-2

Castelle, C. J., Brown, C. T., Thomas, B. C., Williams, K. H., & Banfield, J. F. (2017). Unusual respiratory capacity and nitrogen metabolism in a Parcubacterium (OD1) of the Candidate Phyla Radiation. *Scientific Reports*, *7*(October 2016), 1–12. https://doi.org/10.1038/srep40101

Chaudhari, N. M., Overholt, W. A., Figueroa-Gonzalez, P. A., Taubert, M., Bornemann, T. L. V., Probst, A. J., Hölzer, M., Marz, M., & Küsel, K. (2021). The economical lifestyle of CPR bacteria in groundwater allows little preference for environmental drivers. *BioRxiv*, 2021.07.28.454184. https://www.biorxiv.org/content/10.1101/2021.07.28.454184v1%0Ahttps://www.biorxiv.org/content/10.1101/2021.07.28.454184v1.abstract

Chaumeil, P.-A., Mussig, A. J., Hugenholtz, P., & Parks, D. H. (2020). GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. *Bioinformatics*, *36*(6), 1925–1927. https://doi.org/10.1093/bioinformatics/btz848

Choi, I. G., & Kim, S. H. (2007). Global extent of horizontal gene transfer. *Proceedings of the National Academy of Sciences of the United States of*

America, *104*(11), 4489–4494. https://doi.org/10.1073/pnas.0611557104

Close, M., Abraham, P., Webber, J., Cowey, E., Humphries, B., Fenwick, G., Howard, S., Huynh, K., Grace, T., Dupont, P. Y., & Weaver, L. (2020). Use of Sonication for Enhanced Sampling of Attached Microbes from Groundwater Systems. *Groundwater*, *58*, 901–915. https://doi.org/10.1111/gwat.12984

Cohen, O., Gophna, U., & Pupko, T. (2011). The complexity hypothesis revisited: Connectivity Rather Than function constitutes a barrier to horizontal gene transfer. *Molecular Biology and Evolution*, *28*(4). https://doi.org/10.1093/molbev/msq333

Cordero, O. X., & Hogeweg, P. (2009). The impact of long-distance horizontal gene transfer on prokaryotic genome size. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(51), 21748–21753. https://doi.org/10.1073/pnas.0907584106

Demain, A. L., & Fang, A. (2000). The natural functions of secondary metabolites. In *Advances in biochemical engineering/biotechnology* (Vol. 69). https://doi.org/10.1007/3-540-44964-7_1

Dong, X., Zhang, C., Li, W., Weng, S., Song, W., Li, J., & Wang, Y. (2021). Functional diversity of microbial communities in inactive seafloor sulfide deposits. *FEMS Microbiology Ecology*, *97*(8), 1–10. https://doi.org/10.1093/femsec/fiab108

Du, D., van Veen, H. W., Murakami, S., Pos, K. M., & Luisi, B. F. (2015). Structure, mechanism and cooperation of bacterial multidrug transporters. In *Current Opinion in Structural Biology* (Vol. 33). https://doi.org/10.1016/j.sbi.2015.07.015

Dunning Hotopp, J. C., Clark, M. E., Oliveira, D. C. S. G., Foster, J. M., Fischer, P., Muñoz Torres, M. C., Giebel, J. D., Kumar, N., Ishmael, N., Wang, S., Ingram,

J., Nene, R. V., Shepard, J., Tomkins, J., Richards, S., Spiro, D. J., Ghedin, E., Slatko, B. E., Tettelin, H., & Werren, J. H. (2007). Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. *Science*, *317*(5845). https://doi.org/10.1126/science.1142490

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, *26*, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Emms, D. M., & Kelly, S. (2019). OrthoFinder: Phylogenetic orthology inference for comparative genomics. *Genome Biology*, *20*(1). https://doi.org/10.1186/s13059-019-1832-y

Flynn, T. M., Sanford, R. A., & Bethke, C. M. (2008). Attached and suspended microbial communities in a pristine confined aquifer. *Water Resources Research*, *44*(7), 1–7. https://doi.org/10.1029/2007WR006633

Gabaldón, T., & Huynen, M. A. (2007). From endosymbiont to host-controlled organelle: The hijacking of mitochondrial protein synthesis and metabolism. *PLoS Computational Biology*, *3*(11). https://doi.org/10.1371/journal.pcbi.0030219

Garcia-Vallvé, S., Romeu, A., & Palau, J. (2000). Horizontal gene transfer in bacterial and archaeal complete genomes. *Genome Research*, *10*(11). https://doi.org/10.1101/gr.130000

Geesink, P., Wegner, C.-E., Probst, A. J., Herrmann, M., Dam, H. T., Kaster, A.-K., & Küsel, K. (2020). Genome-inferred spatio-temporal resolution of an uncultivated Roizmanbacterium reveals its ecological preferences in groundwater. *Environmental Microbiology*, *22*(2), 726–737. https://doi.org/https://doi.org/10.1111/1462-2920.14865

Gevers, D., Vandepoele, K., Simillion, C., & Van De Peer, Y. (2004). Gene

duplication and biased functional retention of paralogs in bacterial genomes. In *Trends in Microbiology* (Vol. 12, Issue 4). https://doi.org/10.1016/j.tim.2004.02.007

Giovannoni, S. J., Cameron Thrash, J., & Temperton, B. (2014). Implications of streamlining theory for microbial ecology. In *ISME Journal* (Vol. 8, Issue 8). https://doi.org/10.1038/ismej.2014.60

Gogarten, J. P., Doolittle, W. F., & Lawrence, J. G. (2002). Prokaryotic evolution in light of gene transfer. *Molecular Biology and Evolution*, *19*(12). https://doi.org/10.1093/oxfordjournals.molbev.a004046

Griebler, C., & Lueders, T. (2009). Microbial biodiversity in groundwater ecosystems. *Freshwater Biology*, *54*(4), 649–677. https://doi.org/10.1111/j.1365-2427.2008.02013.x

Gu, Z., Gu, L., Eils, R., Schlesner, M., & Brors, B. (2014). Circlize implements and enhances circular visualization in R. *Bioinformatics*, *30*(19). https://doi.org/10.1093/bioinformatics/btu393

Haft, D. H., Selengut, J. D., & White, O. (2003). The TIGRFAMs database of protein families. In *Nucleic Acids Research* (Vol. 31, Issue 1). https://doi.org/10.1093/nar/gkg128

He, C., Keren, R., Whittaker, M., Farag, I. F., Doudna, J., Cate, J. H. D., & Banfield, J. (2021). Genome-resolved metagenomics reveals site-specific diversity of episymbiotic CPR bacteria and DPANN archaea in groundwater ecosystems. *Nature Microbiology*, *6*, 354–365. https://doi.org/10.1101/2020.05.14.094862

Herrmann, M., Wegner, C.-E., Taubert, M., Geesink, P., Lehmann, K., Yan, L., Lehmann, R., Totsche, K. U., & Küsel, K. (2019). Predominance of Cand. Patescibacteria in Groundwater Is Caused by Their Preferential Mobilization

From Soils and Flourishing Under Oligotrophic Conditions. *Frontiers in Microbiology*, *10*(June), 1–15. https://doi.org/10.3389/fmicb.2019.01407

Hooper, S. D., & Berg, O. G. (2003). Duplication is more common among laterally transferred genes than among indigenous genes. *Genome Biology*, *4*(8). https://doi.org/10.1186/gb-2003-4-8-r48

Hsiao, W. W. L., Ung, K., Aeschliman, D., Bryan, J., Brett Finlay, B., & Brinkman, F. S. L. (2005). Evidence of a large novel gene pool associated with prokaryotic genomic Islands. *PLoS Genetics*, *1*(5). https://doi.org/10.1371 /journal.pgen.0010062

Huerta-Cepas, J., Forslund, K., Coelho, L. P., Szklarczyk, D., Jensen, L. J., von Mering, C., & Bork, P. (2017). Fast Genome-Wide Functional Annotation through Orthology Assignment by eggNOG-Mapper. *Molecular Biology and Evolution*, *34*(8), 2115–2122. https://doi.org/10.1093/molbev/msx148

Huerta-Cepas, J., Szklarczyk, D., Heller, D., Hernández-Plaza, A., Forslund, S. K., Cook, H., Mende, D. R., Letunic, I., Rattei, T., Jensen, L. J., von Mering, C., & Bork, P. (2019). eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. *Nucleic Acids Research*, *47*(D1), D309–D314. https://doi.org/10.1093/nar/gky1085

Hug, L. A., Thomas, B. C., Brown, C. T., Frischkorn, K. R., Williams, K. H., Tringe, S. G., & Banfield, J. F. (2015). Aquifer environment selects for microbial species cohorts in sediment and groundwater. *ISME Journal*, *9*(8), 1846–1856. https://doi.org/10.1038/ismej.2015.2

Hyatt, D., Chen, G.-L., Locascio, P. F., Land, M. L., Larimer, F. W., & Hauser, L. J. (2010). Prodigal: prokaryotic gene recognition and translation initiation site

identification. *BMC Bioinformatics*, *11*, 119. https://doi.org/10.1186/1471-2105-11-119

Jaffe, A. L., Corel, E., Pathmanathan, J. S., Lopez, P., & Bapteste, E. (2016). Bipartite graph analyses reveal interdomain LGT involving ultrasmall prokaryotes and their divergent, membrane-related proteins. *Environmental Microbiology*, *18*(12), 5072–5081. https://doi.org/10.1111/1462-2920.13477

Jain, R., Rivera, M. C., & Lake, J. A. (1999). Horizontal gene transfer among genomes: The complexity hypothesis. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(7). https://doi.org/10.1073/pnas.96.7.3801

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A., & Jermiin, L. S. (2017). ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods*, *14*(6), 587–589. https://doi.org/10.1038/nmeth.4285

Kanehisa, M., Sato, Y., Kawashima, M., Furumichi, M., & Tanabe, M. (2016). KEGG as a reference resource for gene and protein annotation. *Nucleic Acids Research*, *44*(D1), D457–D462. https://doi.org/10.1093/nar/gkv1070

Kanhere, A., & Vingron, M. (2009). Horizontal gene transfers in prokaryotes show differential preferences for metabolic and translational genes. *BMC Evolutionary Biology*, *9*(1), 1–13. https://doi.org/10.1186/1471-2148-9-9

Kieft, K., Zhou, Z., & Anantharaman, K. (2020). VIBRANT: automated recovery, annotation and curation of microbial viruses, and evaluation of viral community function from genomic sequences. *Microbiome*, *8*(1), 90. https://doi.org/10.1186/s40168-020-00867-0

Kondrashov, F. A., Rogozin, I. B., Wolf, Y. I., & Koonin, E. V. (2002). Selection in the evolution of gene duplications. *Genome Biology*, *3*(2).

https://doi.org/10.1186/gb-2002-3-2-research0008

Konstantinidis, K. T., & Tiedje, J. M. (2004). Trends between gene content and genome size in prokaryotic species with larger genomes. *Proceedings of the National Academy of Sciences*, *101*(9), 3160–3165. https://doi.org/10.1073/pnas.0308653100

Koonin, E. V., Makarova, K. S., & Wolf, Y. I. (2017). Evolutionary Genomics of Defense Systems in Archaea and Bacteria. *Annual Review of Microbiology*, *71*. https://doi.org/10.1146/annurev-micro-090816-093830

Langmead, B., Trapnell, C., Pop, M., & Salzberg, S. L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biology*, *10*(3), R25. https://doi.org/10.1186/gb-2009-10-3-r25

Lawrence, J. G., & Ochman, H. (1997). Amelioration of bacterial genomes: Rates of change and exchange. *Journal of Molecular Evolution*, *44*(4). https://doi.org/10.1007/PL00006158

León-Zayas, R., Peoples, L., Biddle, J. F., Podell, S., Novotny, M., Cameron, J., Lasken, R. S., & Bartlett, D. H. (2017). The metabolic potential of the single cell genomes obtained from the Challenger Deep, Mariana Trench within the candidate superphylum Parcubacteria (OD1). *Environmental Microbiology*, *19*(7), 2769–2784. https://doi.org/10.1111/1462-2920.13789

Letunic, I., & Bork, P. (2007). Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. *Bioinformatics (Oxford, England)*, *23*(1), 127–128. https://doi.org/10.1093/bioinformatics/btl529

Lynch, M., & Conery, J. S. (2000). The evolutionary fate and consequences of duplicate genes. *Science*, *290*(5494). https://doi.org/10.1126/science.290.5494.1151

Maddocks, S. E., & Oyston, P. C. F. (2008). Structure and function of the LysR-type transcriptional regulator (LTTR) family proteins. *Microbiology*, *154*(12), 3609–3623. https://doi.org/10.1099/mic.0.2008/022772-0

Majewski, J., & Cohan, F. M. (1999). DNA sequence similarity requirements for interspecific recombination in Bacillus. *Genetics*, *153*(4). https://doi.org/10.1093/genetics/153.4.1525

Majewski, J., Zawadzki, P., Pickerill, P., Cohan, F. M., & Dowson, C. G. (2000). Barriers to genetic exchange between bacterial species: Streptococcus pneumoniae transformation. *Journal of Bacteriology*, *182*(4). https://doi.org/10.1128/JB.182.4.1016-1023.2000

Mandel, M. J., Wollenberg, M. S., Stabb, E. V., Visick, K. L., & Ruby, E. G. (2009). A single regulatory gene is sufficient to alter bacterial host range. *Nature*, *458*(7235). https://doi.org/10.1038/nature07660

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal; Vol 17, No 1: Next Generation Sequencing Data Analysis*. https://doi.org/10.14806/ej.17.1.200

Méheust, R., Burstein, D., Castelle, C. J., & Banfield, J. F. (2019). The distinction of CPR bacteria from other bacteria based on protein family content. *Nature Communications*, *10*(1), 4173. https://doi.org/10.1038/s41467-019-12171-z

Moran, N. A., & Bennett, G. M. (2014). The Tiniest Tiny Genomes. *Annual Review of Microbiology*, *68*(1), 195–215. https://doi.org/10.1146/annurev-micro-091213-112901

Morris, J. J., Lenski, R. E., & Zinser, E. R. (2012). The black queen hypothesis: Evolution of dependencies through adaptive gene loss. *MBio*, *3*(2), 1–7. https://doi.org/10.1128/mBio.00036-12

Mosley, O. E., Gios, E., Weaver, L., Close, M., Daughney, C., van der Raaij, R., Martindale, H., & Handley, K. M. (2022). Metabolic Diversity and Aero-Tolerance in Anammox Bacteria from Geochemically Distinct Aquifers. *MSystems*, *7*(1), 2021.09.16.460709. https://doi.org/10.1128/msystems.01255-21

Murray, N. E. (2000). Type I Restriction Systems: Sophisticated Molecular Machines (a Legacy of Bertani and Weigle). *Microbiology and Molecular Biology Reviews*, *64*(2). https://doi.org/10.1128/mmbr.64.2.412-434.2000

Nakamura, Y., Itoh, T., Matsuda, H., & Gojobori, T. (2004). Biased biological functions of horizontally-transferred genes in prokaryotic genomes. *Nature Genetics*, *36*(7), 760–766. https://doi.org/10.1038/ng1381

Nayfach, S., Camargo, A. P., Schulz, F., Eloe-Fadrosh, E., Roux, S., & Kyrpides, N. C. (2021). CheckV assesses the quality and completeness of metagenome-assembled viral genomes. *Nature Biotechnology*, *39*(5). https://doi.org/10.1038/s41587-020-00774-7

Nelson, W. C., & Stegen, J. C. (2015). The reduced genomes of Parcubacteria (OD1) contain signatures of a symbiotic lifestyle. *Frontiers in Microbiology*, *6*(JUN), 1–14. https://doi.org/10.3389/fmicb.2015.00713

Nguyen, L.-T., Schmidt, H. A., von Haeseler, A., & Minh, B. Q. (2015). IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Molecular Biology and Evolution*, *32*(1), 268–274. https://doi.org/10.1093/molbev/msu300

Nguyen, M. T. H. D., Liu, M., & Thomas, T. (2014). Ankyrin-repeat proteins from sponge symbionts modulate amoebal phagocytosis. *Molecular Ecology*, *23*(6). https://doi.org/10.1111/mec.12384

Nicolas, A. M., Jaffe, A. L., Nuccio, E. E., Taga, M. E., Firestone, M. K., & Banfield,

J. F. (2021). Soil Candidate Phyla Radiation Bacteria Encode Components of Aerobic Metabolism and Co-occur with Nanoarchaea in the Rare Biosphere of Rhizosphere Grassland Communities. *MSystems*, *6*(4). https://doi.org/10.1128/msystems.01205-20

Ochman, Howard, Lawrence, Jeffrey G., & Groisman, Eduardo A. (2000). Lateral gene transfer and the nature of bacterial innovation. *Nature*, *405*(6784), 299–304. http://adsabs.harvard.edu/abs/2000Natur.405..299O

Oliveira, P. H., Touchon, M., & Rocha, E. P. C. (2016). Regulation of genetic flux between bacteria by restriction-modification systems. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(20). https://doi.org/10.1073/pnas.1603257113

Parks, D. H., Chuvochina, M., Chaumeil, P. A., Rinke, C., Mussig, A. J., & Hugenholtz, P. (2020). A complete domain-to-species taxonomy for Bacteria and Archaea. *Nature Biotechnology*, *38*(September). https://doi.org/10.1038/s41587-020-0501-8

Parks, D. H., Imelfort, M., Skennerton, C. T., Hugenholtz, P., & Tyson, G. W. (2015). CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Research*, *25*(7), 1043–1055. https://doi.org/10.1101/gr.186072.114

Parks, D. H., Rinke, C., Chuvochina, M., Chaumeil, P. A., Woodcroft, B. J., Evans, P. N., Hugenholtz, P., & Tyson, G. W. (2017). Recovery of nearly 8,000 metagenome-assembled genomes substantially expands the tree of life. *Nature Microbiology*, *2*(11). https://doi.org/10.1038/s41564-017-0012-7

Potter, S. C., Luciani, A., Eddy, S. R., Park, Y., Lopez, R., & Finn, R. D. (2018). HMMER web server: 2018 update. *Nucleic Acids Research*, *46*(W1).

https://doi.org/10.1093/nar/gky448

Probst, A. J., Elling, F. J., Castelle, C. J., Zhu, Q., Elvert, M., Birarda, G., Holman, H. Y. N., Lane, K. R., Ladd, B., Ryan, M. C., Woyke, T., Hinrichs, K. U., & Banfield, J. F. (2020). Lipid analysis of CO2-rich subsurface aquifers suggests an autotrophy-based deep biosphere with lysolipids enriched in CPR bacteria. *ISME Journal*, *14*(6). https://doi.org/10.1038/s41396-020-0624-4

Probst, A. J., Ladd, B., Jarett, J. K., Geller-McGrath, D. E., Sieber, C. M. K., Emerson, J. B., Anantharaman, K., Thomas, B. C., Malmstrom, R. R., Stieglmeier, M., Klingl, A., Woyke, T., Ryan, M. C., & Banfield, J. F. (2018). Differential depth distribution of microbial function and putative symbionts through sediment-hosted aquifers in the deep terrestrial subsurface. *Nature Microbiology*, *5*, 328–336. https://doi.org/10.1038/s41564-017-0098-y

Rawat, M., & Maupin-Furlow, J. A. (2020). Redox and thiols in archaea. In *Antioxidants* (Vol. 9, Issue 5). https://doi.org/10.3390/antiox9050381

Schulz, F., Eloe-Fadrosh, E. A., Bowers, R. M., Jarett, J., Nielsen, T., Ivanova, N. N., Kyrpides, N. C., & Woyke, T. (2017). Towards a balanced view of the bacterial tree of life. *Microbiome*, *5*(1). https://doi.org/10.1186/s40168-017-0360-9

Sharrar, A. M., Crits-Christoph, A., Méheust, R., Diamond, S., Starr, E. P., & Banfield, J. F. (2020). Bacterial secondary metabolite biosynthetic potential in soil varies with phylum, depth, and vegetation type. *MBio*, *11*(3). https://doi.org/10.1128/MBIO.00416-20

Shen, B. A., & Landick, R. (2019). Transcription of Bacterial Chromatin. In *Journal of Molecular Biology* (Vol. 431, Issue 20). https://doi.org/10.1016/j.jmb.2019.05.041

Shen, P., & Huang, H. V. (1986). Homologous recombination in Escherichia coli: dependence on substrate length and homology. *Genetics*, *112*(3).

https://doi.org/10.1093/genetics/112.3.441

Shin, J., Song, Y., Jeong, Y., & Cho, B. K. (2016). Analysis of the core genome and pan-genome of autotrophic acetogenic bacteria. In *Frontiers in Microbiology* (Vol. 7, Issue SEP). https://doi.org/10.3389/fmicb.2016.01531

Song, W., Wemheuer, B., Zhang, S., Steensen, K., & Thomas, T. (2019). MetaCHIP: Community-level horizontal gene transfer identification through the combination of best-match and phylogenetic approaches. *Microbiome*, *7*(1). https://doi.org/10.1186/s40168-019-0649-y

Sonnhammer, E. L. L., Eddy, S. R., Birney, E., Bateman, A., & Durbin, R. (1998). Pfam: Multiple sequence alignments and HMM-profiles of protein domains. *Nucleic Acids Research*, *26*(1). https://doi.org/10.1093/nar/26.1.320

Soucy, S. M., Huang, J., & Gogarten, J. P. (2015). Horizontal gene transfer: Building the web of life. In *Nature Reviews Genetics* (Vol. 16, Issue 8). https://doi.org/10.1038/nrg3962

Stern, A., & Sorek, R. (2011). The phage-host arms race: Shaping the evolution of microbes. In *BioEssays* (Vol. 33, Issue 1). https://doi.org/10.1002/bies.201000071

Suzek, B. E., Wang, Y., Huang, H., McGarvey, P. B., Wu, C. H., & Consortium, U. (2015). UniRef clusters: a comprehensive and scalable alternative for improving sequence similarity searches. *Bioinformatics (Oxford, England)*, *31*(6), 926–932. https://doi.org/10.1093/bioinformatics/btu739

Swan, B. K., Tupper, B., Sczyrba, A., Lauro, F. M., Martinez-Garcia, M., Gonźalez, J. M., Luo, H., Wright, J. J., Landry, Z. C., Hanson, N. W., Thompson, B. P., Poulton, N. J., Schwientek, P., Acinas, S. G., Giovannoni, S. J., Moran, M. A., Hallam, S. J., Cavicchioli, R., Woyke, T., & Stepanauskas, R. (2013). Prevalent

genome streamlining and latitudinal divergence of planktonic bacteria in the surface ocean. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(28). https://doi.org/10.1073/pnas.1304246110

Tang, L., Nogales, E., & Ciferri, C. (2010). Structure and function of SWI/SNF chromatin remodeling complexes and mechanistic implications for transcription. In *Progress in Biophysics and Molecular Biology* (Vol. 102, Issues 2–3). https://doi.org/10.1016/j.pbiomolbio.2010.05.001

Tee, H. S., Waite, D., Payne, L., Middleditch, M., Wood, S., & Handley, K. M. (2020). Tools for successful proliferation: diverse strategies of nutrient acquisition by a benthic cyanobacterium. *ISME Journal*, *14*(8), 2164–2178. https://doi.org/10.1038/s41396-020-0676-5

The UniProt Consortium. (2017). UniProt: the universal protein knowledgebase. *Nucleic Acids Research*, *45*(D1), D158–D169. https://doi.org/10.1093/nar/gkw1099

Touchon, M., Bernheim, A., & Rocha, E. P. C. (2016). Genetic and life-history traits associated with the distribution of prophages in bacteria. *ISME Journal*, *10*(11). https://doi.org/10.1038/ismej.2016.47

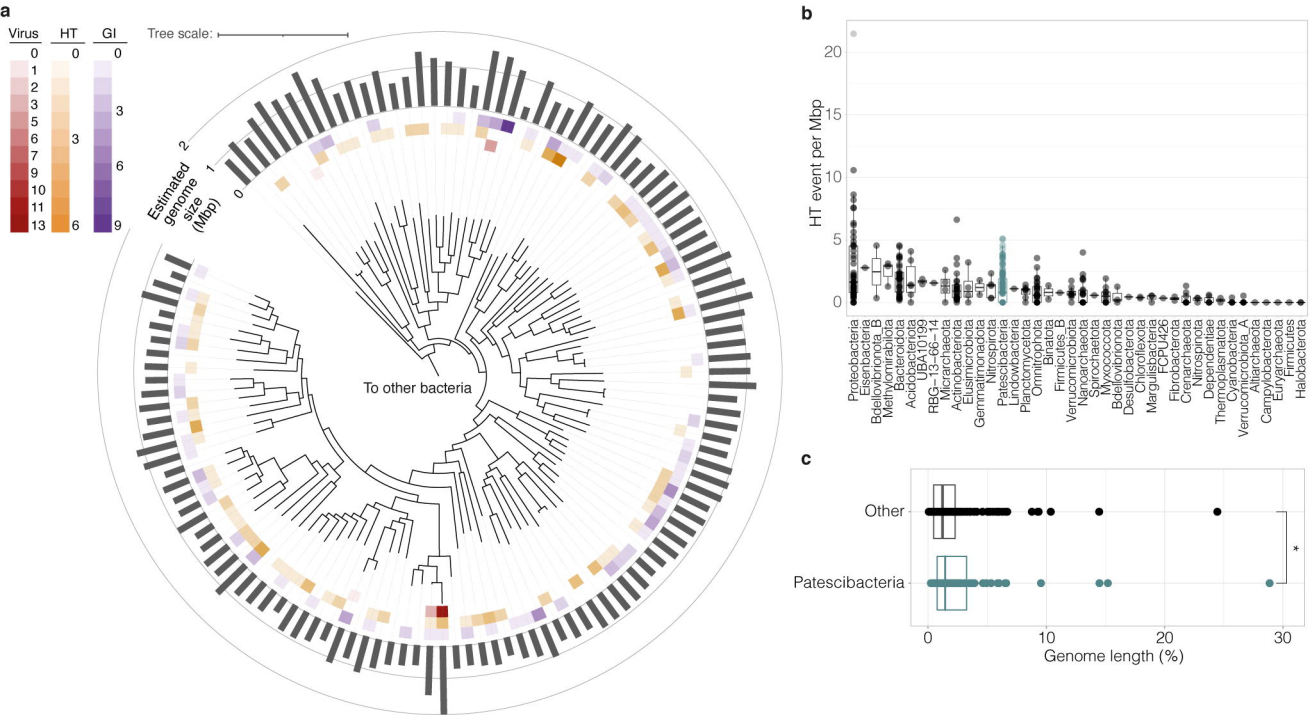
Treangen, T. J., & Rocha, E. P. C. (2011). Horizontal transfer, not duplication, drives the expansion of protein families in prokaryotes. *PLoS Genetics*, *7*(1). https://doi.org/10.1371/journal.pgen.1001284

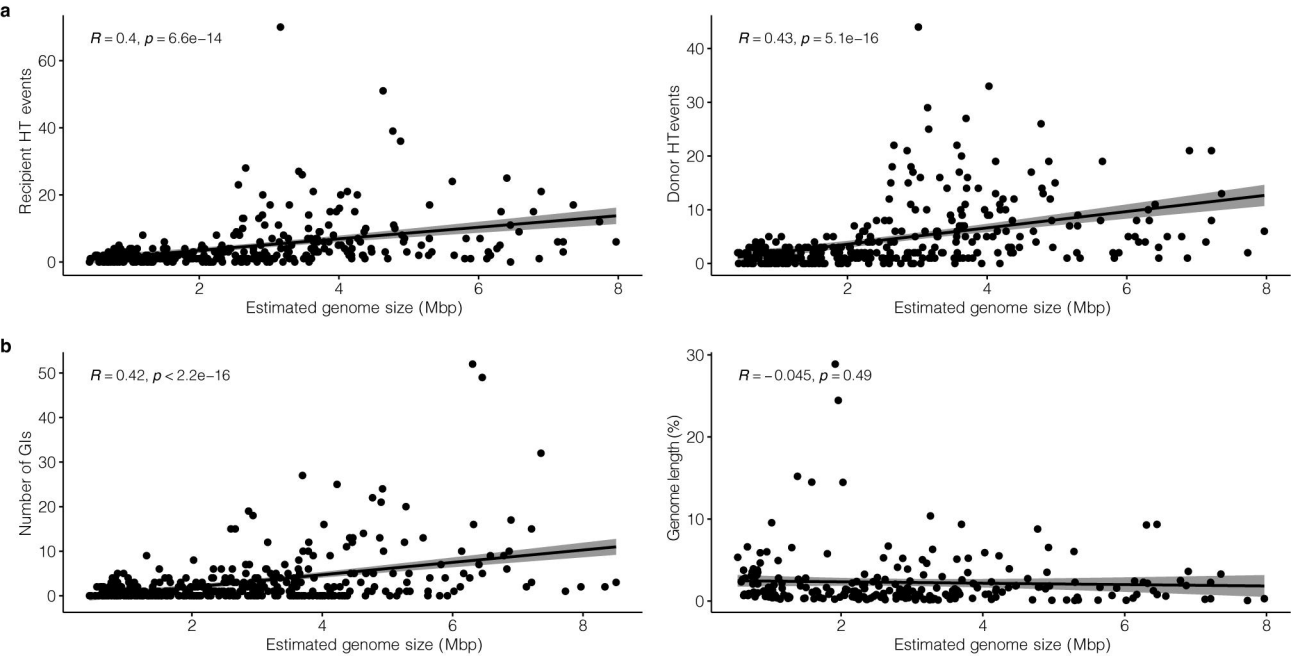
Vasu, K., & Nagaraja, V. (2013). Diverse Functions of Restriction-Modification Systems in Addition to Cellular Defense. *Microbiology and Molecular Biology Reviews*, *77*(1). https://doi.org/10.1128/mmbr.00044-12

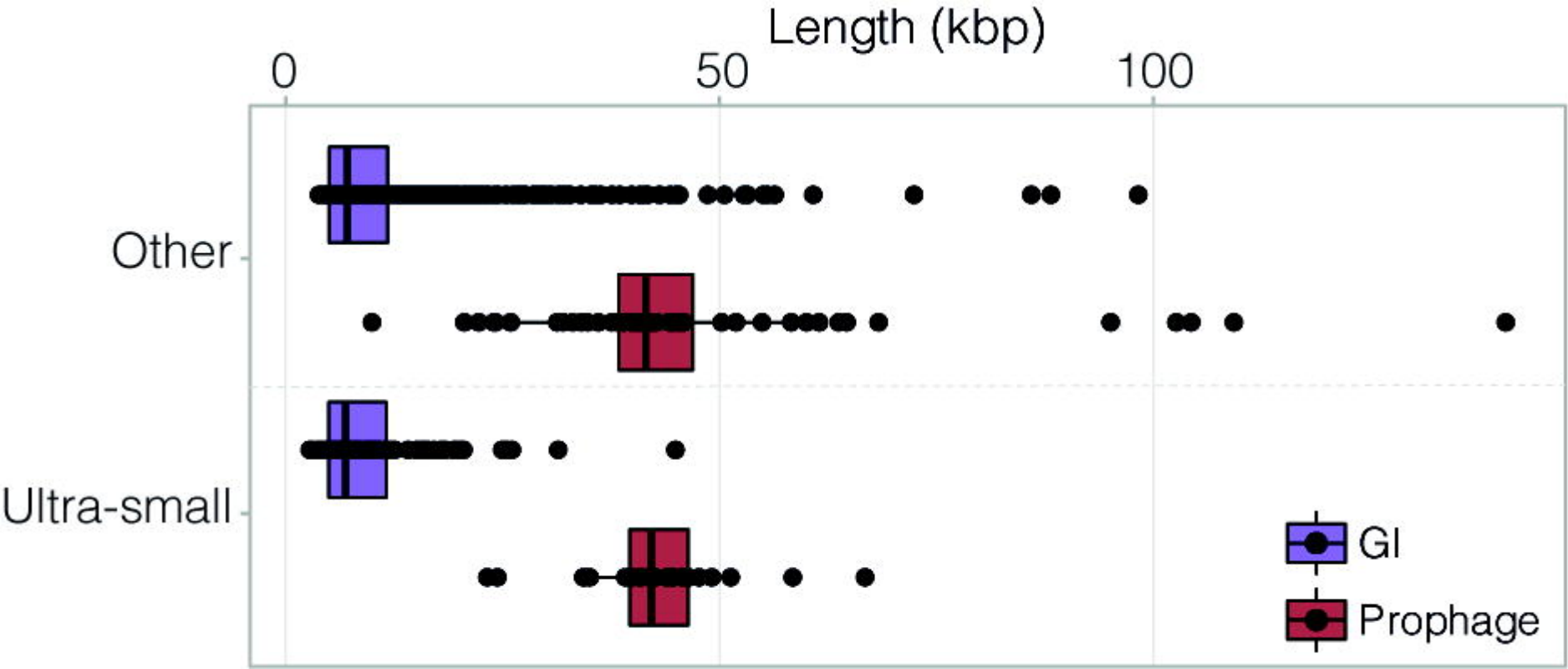
Voeller, D. M., Changchien, L. ming, Maley, G. F., Maley, F., Takechi, T., Turner, R. E., Montfort, W. R., Allegra, C. J., & Chu, E. (1995). Characterization of a

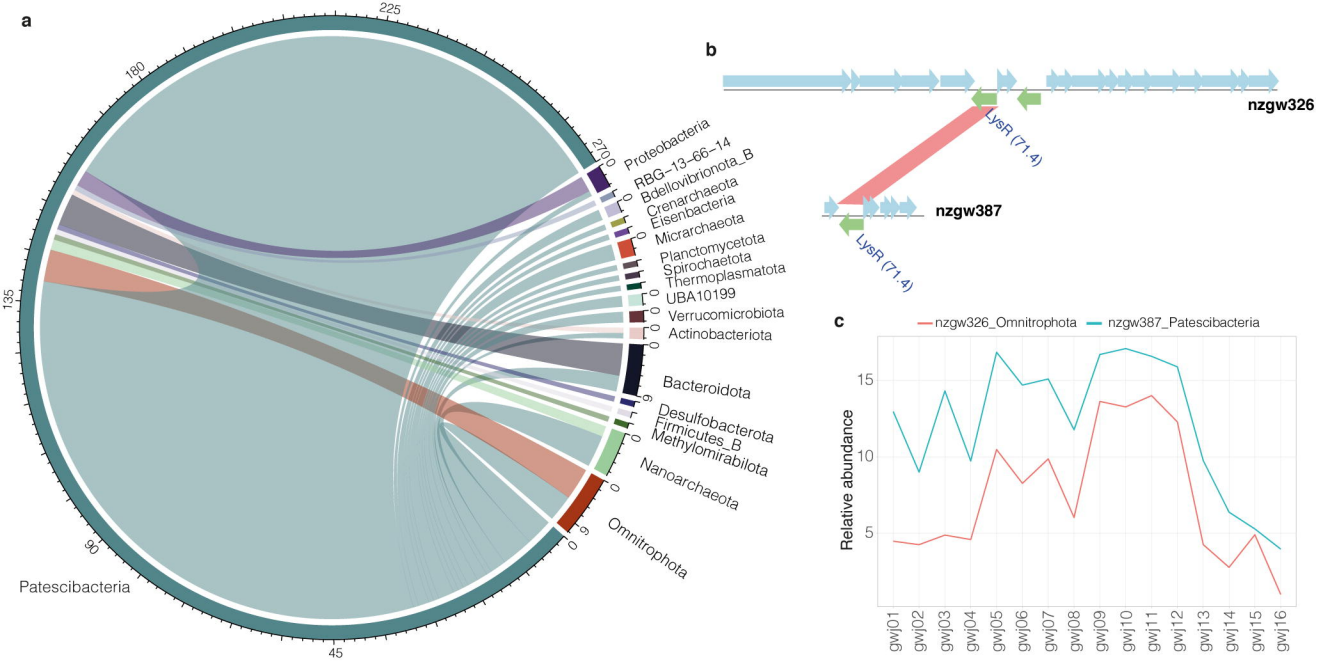
specific interaction between Escherichia coli thymidylate synthase and escherichia coli thymidylate synthase mRNA. *Nucleic Acids Research*, *23*(5). https://doi.org/10.1093/nar/23.5.869

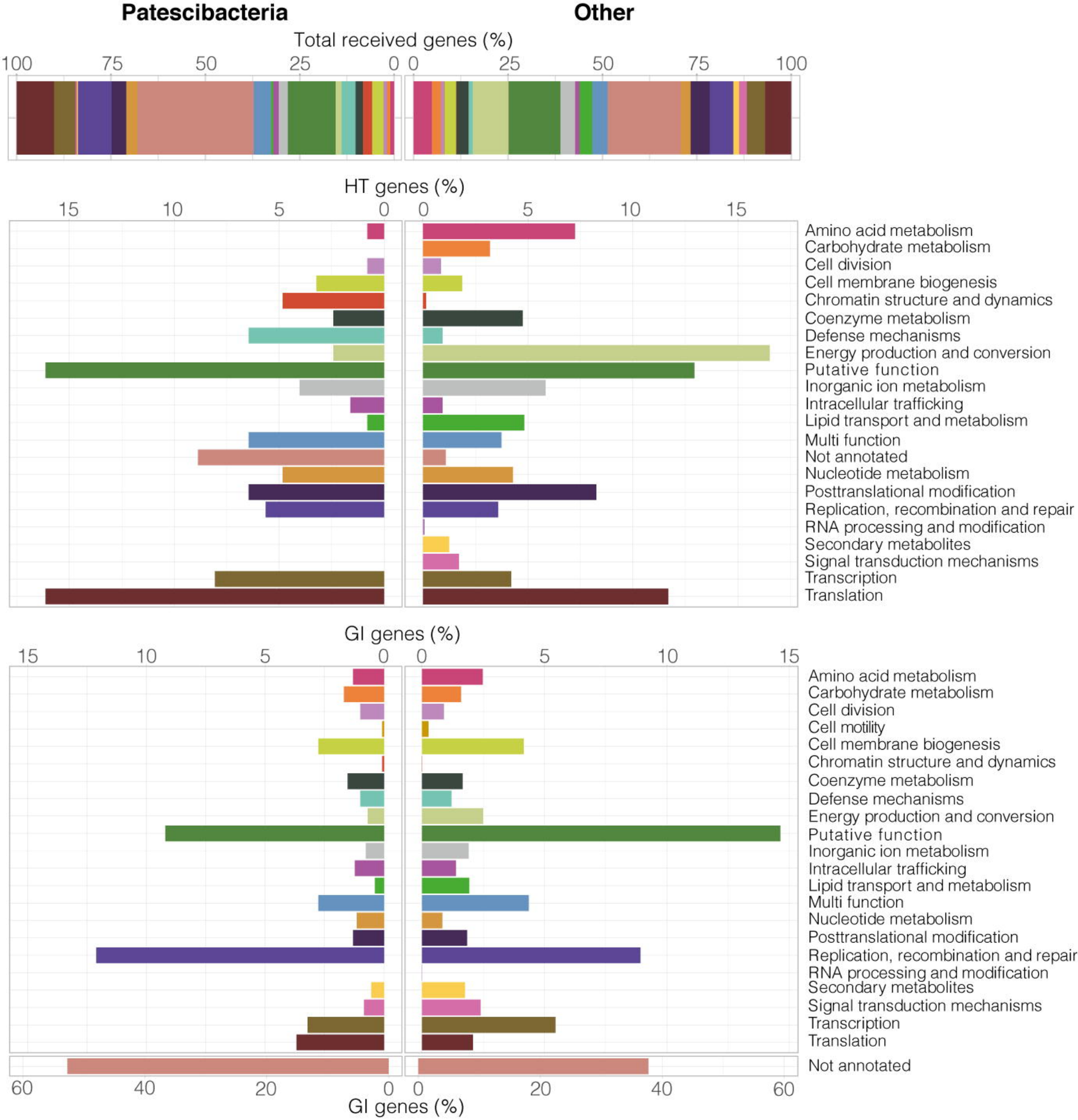
Weigert, M., & Kümmerli, R. (2017). The physical boundaries of public goods cooperation between surface-attached bacterial cells. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1858). https://doi.org/10.1098/rspb.2017.0631

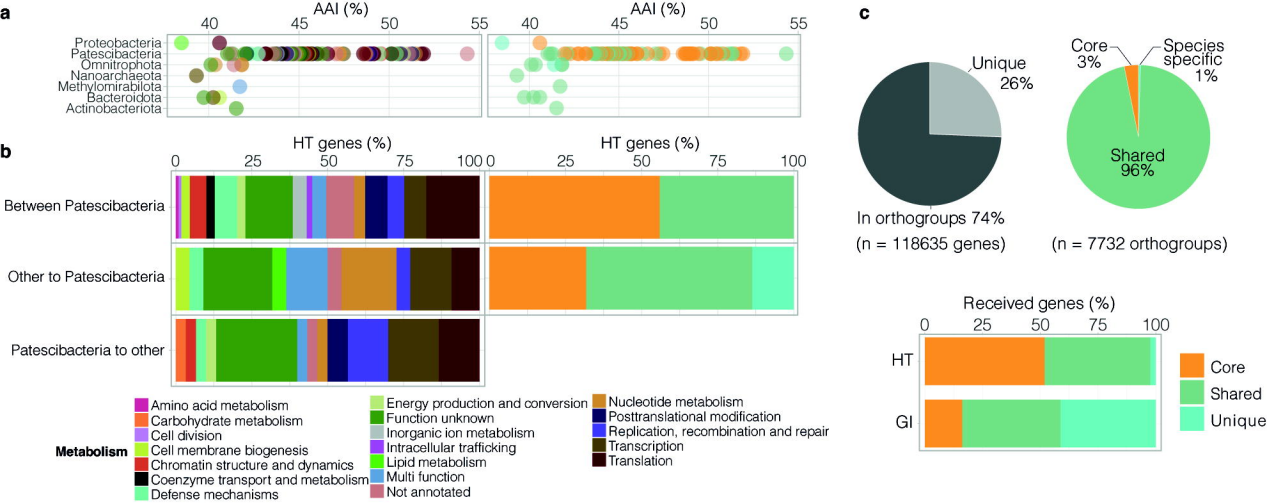

a
Virus
HT
GI
Tree scale:
Estimated genome size (Mbp)
To other bacteria
b
HT event per Mbp
Proteobacteria
Eisenbacteria
Bdellovibrionota_B
Methylomirabilota
Bacteroidota
Acidobacteriota
Dependentiae
RBG−13−66−14
Micrarchaeota
Actinobacteriota
Elusimicrobiota
Gemmatimonadota
Nitrospirota
Patescibacteria
Lindowbacteria
Planctomycetota
Omnitrophota
Bdellovibrionota
Firmicutes_B
Verrucomicrobiota
Nanoarchaeota
Spirochaetota
Myxococcota
Bdellovibrionota
Desulfobacterota
Chloroflexota
Margulisbacteria
CPU426
Fibrobacterota
Crenarchaeota
Nitrospinota
Dependentiae
Thermoplasmatota
Cyanobacteria
Verrucomicrobia_A
Altiarchaeota
Campylobacterota
Euryarchaeota
Firmicutes
Halobacterota
c
Other
Patescibacteria
*
Genome length (%)

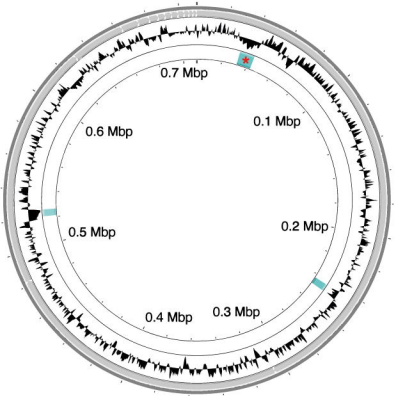

a
R = 0.4, p = 6.6e−14
Recipient HT events
Estimated genome size (Mbp)
R = 0.43, p = 5.1e−16
Donor HT events
Estimated genome size (Mbp)
b
R = 0.42, p < 2.2e−16
Number of GIs
Estimated genome size (Mbp)
R = −0.045, p = 0.49
Genome length (%)
Estimated genome size (Mbp)

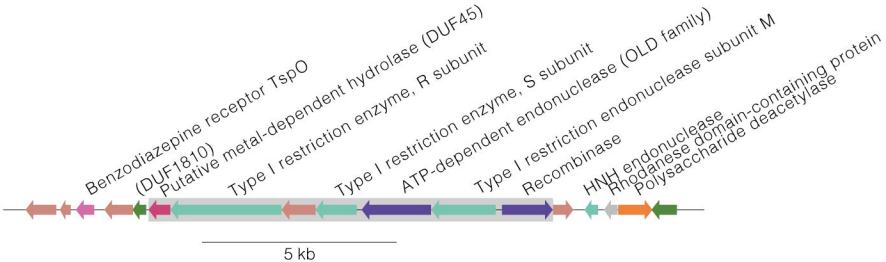

Length (kbp)
0
50
100
Other
Ultra-small
GI
Prophage


a
225
180
135
90
45
270
Patescibacteria
Proteobacteria
RBG-13-66-14
Bdellovibrionota_B
Crenarchaeota
Eisenbacteria
Micrarchaeota
Planctomycetota
Spirochaetota
Thermoplasmatota
UBA10199
Verrucomicrobiota
Actinobacteriota
Bacteroidota
Desulfobacterota
Firmicutes_B
Methylomirabilota
Nanoarchaeota
Omnitrophota
b
nzgw326
LysR (71.4)
nzgw387
LysR (71.4)
c
nzgw326_Omnitrophota
nzgw387_Patescibacteria
Relative abundance
15
10
5
gwj01
gwj02
gwj03
gwj04
gwj05
gwj06
gwj07
gwj08
gwj09
gwj10
gwj11
gwj12
gwj13
gwj14
gwj15
gwj16


Patescibacteria
Other
Total received genes (%)
100
75
50
25
0
0
25
50
75
100
HT genes (%)
15
10
5
0
0
5
10
15
Amino acid metabolism
Carbohydrate metabolism
Cell division
Cell membrane biogenesis
Chromatin structure and dynamics
Coenzyme metabolism
Defense mechanisms
Energy production and conversion
Putative function
Inorganic ion metabolism
Intracellular trafficking
Lipid transport and metabolism
Multi function
Not annotated
Nucleotide metabolism
Posttranslational modification
Replication, recombination and repair
RNA processing and modification
Secondary metabolites
Signal transduction mechanisms
Transcription
Translation
GI genes (%)
15
10
5
0
0
5
10
15
Amino acid metabolism
Carbohydrate metabolism
Cell division
Cell motility
Cell membrane biogenesis
Chromatin structure and dynamics
Coenzyme metabolism
Defense mechanisms
Energy production and conversion
Putative function
Inorganic ion metabolism
Intracellular trafficking
Lipid transport and metabolism
Multi function
Nucleotide metabolism
Posttranslational modification
Replication, recombination and repair
RNA processing and modification
Secondary metabolites
Signal transduction mechanisms
Transcription
Translation
Not annotated
60
40
20
0
0
20
40
60
GI genes (%)


a
AAI (%)
40
45
50
55
Proteobacteria
Patescibacteria
Omnitrophota
Nanoarchaeota
Methylomirabilota
Bacteroidota
Actinobacteriota
b
HT genes (%)
0
25
50
75
100
Between Patescibacteria
Other to Patescibacteria
Patescibacteria to other
Metabolism
Amino acid metabolism
Carbohydrate metabolism
Cell division
Cell membrane biogenesis
Chromatin structure and dynamics
Coenzyme transport and metabolism
Defense mechanisms
Energy production and conversion
Function unknown
Inorganic ion metabolism
Intracellular trafficking
Lipid metabolism
Multi function
Not annotated
Nucleotide metabolism
Posttranslational modification
Replication, recombination and repair
Transcription
Translation
c
Unique
26%
In orthogroups 74%
(n = 118635 genes)
Core
3%
Species
specific
1%
Shared
96%
(n = 7732 orthogroups)
Received genes (%)
HT
GI
Core
Shared
Unique


0.7 Mbp
0.1 Mbp
0.6 Mbp
0.5 Mbp
0.2 Mbp
0.4 Mbp
0.3 Mbp


Benzodiazepine receptor TspO
(DUF1810)
Putative metal-dependent hydrolase (DUF45)
Type I restriction enzyme, R subunit
Type I restriction enzyme, S subunit
ATP-dependent endonuclease (OLD family)
Type I restriction endonuclease subunit M
Recombinase
HNH endonuclease
Rhodanese domain-containing protein
Polysaccharide deacetylase
5 kb
Metabolism
Amino acid metabolism
Carbohydrate metabolism
Defense mechanisms
Putative function
Inorganic ion metabolism
Not annotated
Replication, recombination and repair
Signal transduction mechanisms