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Identifying bacterial functional strategies

Title

Data-driven identification of major axes of functional variation in bacteria

List of authors

Geneviève Lajoie*[1,2,3], Steven W. Kembel[1]

* Email for correspondence (G. Lajoie): genevieve.lajoie@umontreal.ca

[1] Département des Sciences Biologiques, Université du Québec à Montréal, 141 Avenue du

Président-Kennedy, Montréal, Canada, H2X 1Y4

Current address (G. Lajoie) :

[2] Jardin Botanique de Montréal, 4101 rue Sherbrooke E, Montréal, Canada, H1X 2B2

[3] Institut de Recherche en Biologie Végétale, Université de Montréal, 4101 rue Sherbrooke E,

Montréal, Canada, H1X 2B2

Data availability statement

All metagenomic datasets used in this study can be accessed either on the IMG/M

(https://img.jgi.doe.gov/cgi-bin/m/main.cgi) or the MG-RAST (https://www.mg-rast.org/) online

databases using the sample identification information provided in Appendix S1: Table S2. The

functional and phylogenetic genomic datasets can be accessed within the AnnoTree database

(http://annotree.uwaterloo.ca/app/downloads.html) using the sample identification information

provided in Appendix S1: Table S3. The novel R code developed for processing and analyzing

the data is available for review through Figshare (https://figshare.com/s/2d9835e083f160939d66)

and will be made public in a GitHub repository (https://github.com/glajoie1/Bact_Fun_Axes)

upon publication of the manuscript.

Abstract

The discovery of major axes of correlated functional variation among species and habitats

has revealed the fundamental trade-offs structuring both functional and taxonomic diversity in

eukaryotes such as plants. Whether such functional axes exist in the bacterial realm and whether

they could explain bacterial taxonomic turnover among ecosystems remains unknown. Here we

use a data-driven approach to leverage global genomic and metagenomic datasets to reveal the

existence of major axes of functional variation explaining both evolutionary differentiation

within Bacteria and their ecological sorting across diverse habitats. We show that metagenomic

variation among bacterial communities from various ecosystems is structured along a few axes

of correlated functional pathways. Similar clusters of traits explained phylogenetic trait variation

among >16,000 bacterial genomes, suggesting that functional turnover among bacterial

communities from distinct habitats does not only result from the differential filtering of similar

functions among communities, but also from phylogenetic correlations among these functions.

Concordantly, functional pathways associated with trait clusters that were most important for

defining functional turnover among bacterial communities were also those that had the highest

phylogenetic signal in the bacterial genomic phylogeny. This study overall underlines the

important role of evolutionary history in shaping contemporary distributions of bacteria across

ecosystems.

Originality-Significance Statement

In this article, we use a trait screening approach based on genomic and metagenomic data to

identify the key functional strategies of bacteria across ecosystems but also across the bacterial

tree of life. This novel approach allows us to quantify the role of evolutionary processes in

structuring microbial ecological differences among ecosystems. By reducing the high-

dimensionality of trait variation observed among microorganisms around a small number of

fundamental axes of trait covariation, we make a significant step towards generalization of the

drivers of biological diversity in microbes but also across study systems. This research provide a

major advance in our understanding of the origin and maintenance of bacterial biological

diversity, expanding on related findings for plants and animals.

Key Words

Functional strategies, Functional traits, Bacteria, Genomics, Metagenomics, Phylogenetics, Trait

evolution

Introduction

The identification of major axes of variation in life-history strategies among organisms

and habitats has led to advances of both theory and practice in ecology and evolutionary biology

(Grime 1977, Westoby et al. 1998, Lajoie and Kembel 2019). For example, in plants, large-scale

screening of plant functional traits (Westoby 1998, Díaz et al. 2016) led to the discovery that a

few major axes of covarying traits related to leaf economics, seed mass, and plant height explain

most of the global variation in plant functional and life-history strategies. The contribution of

this work has been twofold; first, it improved our understanding of the major environmental

drivers and evolutionary origins of functional variation among organisms (Ackerly and Reich

1999, Wright et al. 2004). Second, the identification of a subset of measurable functional traits

that could be used to characterize ecological strategies helped coordinate measurement efforts

around a reduced set of interpretable functions, thus facilitating attempts at generalization among

study systems worldwide (Díaz et al. 2016, Madani et al. 2018).

Recent conceptual and technological developments in the study of microbes have led to a

proliferation of microbial trait measurements and screenings that have opened the door for

extending this research agenda to the microbial realm. Still, functional categorization schemes

proposed to date in microbial ecology have mostly consisted of direct translations of functional

strategy schemes developed for macro-organisms. Classifications focusing on growth responses

of organisms, such as the r-K selection spectrum or Grime's CSR (competitive, stress-tolerant,

ruderal) scheme (e.g. Fierer et al. 2007, Evans and Wallenstein 2014, Santillan et al. 2019), have

drawn particular interest and been useful in improving our understanding of ecosystem

functioning in certain habitats (Malik et al. 2020). The relevance of such schemes in explaining

global patterns of microbial functional diversity however remains limited by their reliance on in-

vitro phenotypic measurements, still unachievable for a vast diversity of microbial strains (Steen

et al. 2019).

Metagenomic sequencing data represent a pragmatic alternative for the large-scale

characterization of microbial functions. Functional predictions from metagenomic sequences

have been useful for identifying sets of functional genes that best discriminate microbial samples

across ecosystems (Dinsdale et al. 2008, Fierer et al. 2012, Ramírez-Flandes et al. 2019). For

example, genes encoding oxidoreductases were shown to be the most useful of enzyme-encoding

genes in separating metagenomic samples into biome groups (e.g. terrestrial vs. aquatic)

(Ramírez-Flandes et al. 2019). By constraining their analyses along predetermined

environmental axes, these studies allow the identification of functional genes that are

distinctively enriched in each habitat in comparison to others. They however fall short in

determining whether such 'indicator genes' take part in larger strategies that structure functional

variation among microbial communities across diverse habitats – as observed for macro-

organisms – or whether they tend to explain functional variation among habitat types

idiosyncratically.

Objectives and hypotheses

Here, we evaluate whether functional variation among bacterial metagenomes from

different ecosystems is driven by major groups of correlated traits (i.e. functional strategies). We

then compare these groups of traits to those explaining the most variation among a set of

phylogenetically diverse bacterial genomes to test hypotheses about the ecological or

evolutionary origin of these strategies. We predict that if the presence of correlated axes of trait

variation across ecosystems is mostly explained by the filtering of groups of traits differentially

selected along environmental gradients, then the groups of traits uncovered in genomes vs.

metagenomes are unlikely to be the same between datasets (Appendix S1: Table S1). Reversely,

if evolutionary selection or constraints on the variation of these groups of traits through time

contribute to structuring functional variation among metagenomes (Futuyma 2010, Muir 2015),

then the groups of traits should correspond among datasets. We lastly address the contribution of

phylogenetic processes to correlated trait variation across metagenomes by testing if traits

belonging to groups explaining the most variation among metagenomes also have stronger

phylogenetic signal than other traits, and determine the phylogenetic depth at which they are

most structured. Overall, this study contributes to a more general understanding of the role of

functional strategies in driving bacterial distributions across ecosystems and of their evolutionary

origins.

Methods

Metagenomic dataset collection and processing

We searched the IMG/M (Chen et al. 2019) and the MG-RAST (Keegan et al. 2016)

databases for shotgun metagenomic sequence datasets from environmental and host-associated

sources. We restricted our search to types of habitats for which we could obtain data from >1

study and for at least 5 different samples. To limit biases due to sequencing technology (Clooney

et al. 2016), only datasets that had been sequenced with an Illumina system were included. To

uniformize functional annotations among databases, we performed functional and taxonomic

annotations of the IMG/M sequence datasets with the standard MG-RAST annotation pipeline

(Keegan et al. 2016) using BLAST annotation identity criteria of a maximum e-value of 1e-10

and minimum percent identity of 30%, and a minimum sequence length of 125 nucleotides. To

facilitate comparisons with the genomic dataset, metagenomic sequences were filtered to the

bacterial domain only, which represented most annotated sequences (~94% of all taxonomic

annotations). Past this filtering step, we only kept samples that had at least 100 000 sequences

with both functional and taxonomic gene annotations, resulting in a dataset of 114 samples

representing ten major bacterial habitats (Appendix S1: Table S2).

To control for differences in sequencing depth among samples, we randomly picked

100 000 sequences from each sample to generate functional and taxonomic community

composition tables. Rarefying at that level was sufficient to capture the diversity of functional

genes and taxonomic groups present in each sample (Appendix S2: Figure S1). To generate a

functional composition table that had more interpretability and lower dimensionality, we

summed counts of functional genes for each sample by functional pathway (i.e. Tier 3 KEGG

category), the least aggregated of the three levels of the KEGG BRITE gene hierarchy (Kanehisa

et al. 2014). Compositional data was transformed into relative abundance data for each sample.

The resulting functional composition table spanned 245 functional pathways over 114 samples.

A principal coordinates analysis of this dataset constrained by database of origin (i.e. IMG or

MG-RAST) confirmed that database did not drive much variation among samples (1.9% of

variation explained). The relative abundance of each taxon in each sample was similarly

compiled at the phylum, class and order levels using the RefSeq taxonomic classification (Leary

et al. 2016). The final taxonomic datasets spanned 28 phyla, 52 classes, 121 orders and 248

families of bacteria across all samples (Appendix S2: Figure S2).

Genomic dataset collection and processing

The genomic dataset was retrieved from the AnnoTree server (accessed Dec. 2019)

(Mendler et al. 2019). It consists of the functional annotations, taxonomic annotations and

phylogenetic relationships of more than 27,000 fully sequenced bacterial genomes. We

simplified the dataset by randomly retaining a single genome at each tip of the phylogeny,

leading to a final dataset of 15,973 genomes (Appendix S1: Table S3). The taxonomic identity of

all genomes retained spanned 111 phyla, 270 classes, 735 orders and 1659 families. The

taxonomic composition of bacterial genomes in the genomic dataset encompassed the same

major phyla recorded across metagenomes (Appendix S2: Figure S2). Counts of protein-coding

genes for each genome were transformed into relative abundances of KEGG functional pathways

as with the metagenomic dataset. We overall retrieved 312 pathways across genomes.

Identification of main functional axes in the metagenomic dataset

We used a hierarchical clustering approach to identify groups of correlated functions that

could constitute major ecological strategies across metagenomic samples. We first generated a

principal coordinate analysis (PCoA) of the metagenomic functional dataset based on the

Hellinger distance between samples using R package *ape* (Paradis and Schliep 2018). We then

fitted the functional pathways onto the ordination to obtain vectors maximizing the correlation of

these pathways with each ordination axis with R package *vegan* (Oksanen et al. 2013). To

identify correlated axes of functional variation, we then calculated distances between functional

pathways using the coordinates of these vectors across the first four PCoA axes (together

explaining 74% of variance in the ordination). We used the resulting distance matrix to perform a

hierarchical agglomerative clustering of functional pathways using Ward's minimum variance

method (D2) to minimize within-cluster variance. We identified clusters of correlated traits (i.e.

trait clusters) by visual examination of the dendrogram based on their contribution to functional

variation across the first 4 axes of the PCoA. The number of clusters in the dendrogram was

confirmed based on a majority-ruling of 30 indices calculating the optimal number of clusters in

a dendrogram using R package *NbClust* (Charrad et al. 2014). We then tested whether the

number of pathways from each functional category differed among clusters when compared with

their observed prevalence in the dataset using a chi-square test on counts of functional pathways

by Tier 2 functional category in the KEGG classification framework, keeping only Tier 2

categories for which there were at least 10 observations across clusters.

Drivers of functional variation among metagenomes

We evaluated if the richness of functional pathways within any sample varied among

habitats using a type III analysis of variance controlling for unequal sample sizes among factors

(Weisberg 2019). We compared habitat types in pairs using least square means comparisons

using multivariate-*t* corrections with the R package *emmeans* (Lenth 2022). We then tested

whether differences in functional richness among samples explained the functional distances

among samples using a PERMANOVA. We also evaluated how much variation in the functional

composition among samples could be explained by habitat type, taxonomic composition (class-

level) and their interaction using a variation partitioning analysis. These analyses were

performed using R package *vegan* (Oksanen et al. 2013).

Phylogenetic structure of functional variation in the genomic dataset

We assessed the phylogenetic covariation of functional traits by performing a

phylogenetic principal components analysis (pPCA) on the genomic functional dataset, using R

package *adephylo* (Jombart and Dray 2010). This approach finds axes of functional variation that

maximize the product of variance of the scores and their phylogenetic autocorrelation, revealing

axes of correlated trait variation that are phylogenetically structured (Jombart et al. 2010). We

calculated functional distances among pathways using the loadings obtained through this

ordination over the first 4 axes of the pPCA, which encompassed ~65% of explained variance.

We used these distances as described previously to perform a hierarchical clustering analysis of

functional pathways using Ward's minimum variance method.

We compared the structure of functional covariation in the metagenomic and genomic

dataset with a Procrustes analysis performed on the cophenetic distance matrices describing each

of the two hierarchical clusterings. The two dendrograms were pruned beforehand to include the

same functional pathways. The significance of the Procrustes statistic, describing the similarity

between the two datasets, was assessed by comparing the observed statistic to a distribution of

999 statistics generated through permutations of the original data (Oksanen et al. 2013). We

generated a tanglegram of the two clusterings for visual comparison of their respective functional

groupings. The trees were untangled prior to plotting using R package *dendextend* (Galili 2015).

Phylogenetic signal and depth of ecologically important functions

We next evaluated whether pathways that drove functional differences among ecosystems

tended to have high phylogenetic signal and to vary at more recent or ancient scales in the

bacterial phylogeny. To test this, we calculated the phylogenetic signal of all functional

pathways at increasing depths in the phylogeny using a phylogenetic correlogram. Confidence

intervals were estimated through bootstrapping (n=100) of autocorrelation estimates after re-

standardizations of phylogenetic weights (Keck et al. 2016). We then tested the effect of cluster

affiliation, phylogenetic depth, and their interaction on the phylogenetic signal of traits using a

Type III ANOVA for unbalanced designs. Differences among pairs of groups were tested at each

depth using contrasts on estimated marginal means, as implemented in R package *emmeans*

(Lenth 2022). We also tested whether the loadings of each pathway in the metagenomic PCoA

was correlated with the strength of phylogenetic signal (absolute value of the signal) at each of 5

different depths using a linear model for each of the first four PCoA dimensions.

Results

Main strategies driving functional variation among metagenomes

Four major axes of functional variation explained nearly 75% of variance in the

composition of functional pathways among metagenomic samples (Figure 1). These axes

corresponded to functional transitions among major types of habitats, namely between animal

guts, plants and soil, and aquatic habitats across the first 2 axes of variation (Figure 1ab). The

third axis was characterized by functional variation between soil and aquatic sediment bacterial

communities and saltwater ones, while the transition from (i) free-living and epiphytic habitats to

(ii) endophytic ones characterized the fourth axis. Functional pathways could be divided into 9

main groups of correlated traits based on their contribution to functional variation across these

axes (Figure 2a, Appendix S2: Figure S3, Appendix S1: Table S4). The proportions of functional

categories to which the functional pathways belonged also differed significantly among groups

(Tier-1 categories: X^2(32, N=246) = 110.6, p<0.001; Tier-2 categories: X^2(216, N=246) =

397.62, p<0.001) (Figure 2b).

Habitat type explained an important portion of turnover in functional composition among

samples (PERMANOVA: $F_{(9,104)} = 11.917$, $p=0.001$, $R^2 = 51\%$). The functional composition

of trait clusters can thus be described in relation to the habitats with which they are most

associated. Namely, a cluster (C2) composed of traits pertaining to genetic information

processing (i.e. replication and repair; translation; folding sorting and degradation) and

nucleotide metabolism explained the functional differentiation of animal gut samples as

compared with soil and plants samples (Axis 1). A cluster (C9) characterized by traits related to

xenobiotics biodegradation and metabolism, amino acid metabolism as well as cell growth and

death explained the variation in the opposite direction. Along the second axis, functional

pathways (C6) participating in carbohydrate metabolism, energy metabolism (i.e.

photosynthesis) and the metabolism of cofactors and vitamins were together enriched in samples

from aquatic communities, while pathways (C1) participating in glycan biosynthesis and

metabolism, metabolism of other amino acids and cell motility were enriched in terrestrial ones

(both free-living and host-associated).

A cluster of traits (C4) involved predominantly in the metabolism of cofactors and

vitamins and in environmental information processing (i.e. signal transduction) explained

variation along the third axis and were enriched in the saltwater communities as opposed to the

soil one. No clear predominance of any functional group emerges for the cluster (C5) correlated

with the opposite side of this axis. Compared to other clusters, those (C3, C8) driving variation

along the 4th axis both contained pathways involved in prokaryote community sensing and cell-

cell interactions. In contrast with cluster C8 associated with endophytic Porifera and plant

microbiomes, the cluster (C3) correlated with non-endophytic communities was also enriched in

functions related to the biosynthesis of other secondary metabolites.

While functional differences among samples could be linked to the turnover in the

identity of pathways that characterize these communities, differences in the richness of

functional pathways among samples explained 14% of variation in functional distances among

samples in a constrained ordination. Indeed, the functional diversity of individual samples varied

among bacterial habitats, as revealed for both richness (Type-III ANOVA: $F_{(9,104)} = 7.855$,

$p<0.001$) and Shannon diversity (Type-III ANOVA: $F_{(9,104)} = 7.695$, $p<0.001$). Bacterial

communities of animal guts (e.g. mammal, bird and insect guts) were generally the least

functionally diverse while communities associated with plants (e.g. vascular plants and

bryophytes) and soil were the most functionally diverse (Appendix S2: Figure S4). Controlling

for the effect of richness on the PCoA however did not affect to a large extent the configuration

of samples in the ordination (m12 squared $= 0.5071$, corr. $= 0.7020$, sig. $= 0.001$, # permutations

$= 999$). Most variation in the functional composition of samples among habitats could be

explained by turnover in the taxonomic composition of these communities (Figure 1bd,

Appendix S2: Figure S5).

Phylogenetic structure of correlated trait variation

The first four axes of the phylogenetic PCA explained 65% of phylogenetic variation in

the genomic functional dataset. Clusters of functional pathways that were important in

explaining functional variation among ecosystems were also important in explaining

phylogenetic variation in the functional composition of genomes (m12 squared $= 0.3618$, corr. $=$

0.7989, sig. $= 0.001$, # permutations $= 999$) (Figure 3). Across all traits, the intensity of

phylogenetic signal varied significantly as a function of depth, with higher signal at lower depths

and lowest signal at intermediate depths. While the signal was positive at lowest depths, it

became negative about mid-way towards the root of the tree. The strength of phylogenetic signal

also varied significantly among metagenomic functional clusters and in interaction with

phylogenetic depth (Figure 4, Appendix S1: Table S5). Clusters 2 and 9 exhibited more

phylogenetic signal than other clusters at low phylogenetic depths, while clusters 1,5 and 7

exhibited the least. The latter clusters were also the least phylogenetically structured at deeper

depths, while cluster 6 and cluster 2 were the most structured. These differences can be linked

with variation in the mean phylogenetic depths of traits belonging to different Tier 1 category,

with genetic information processing pathways (enriched in Cluster 2) showing the most signal

across phylogenetic depths (Appendix S2: Figure S6). Functional pathways that contributed most

to variation among metagenomic samples along the first PCoA axis (characterized by a trade-off

between clusters 2 and 9) also consistently displayed the strongest signal in the genomic

phylogeny (Appendix S2: Figure S7, Appendix S1: Table S6). This relationship was observed

across most depths but was stronger at low phylogenetic depths. It was weaker or null across the

other PCoA dimensions.

Discussion

In this study, we show that functional variation among bacterial communities sampled

across diverse habitats is structured by clusters of correlated functions defining contrasting life-

history strategies among major bacterial habitat types. The structure of these clusters derived

from metagenomic annotation data was similar to that obtained from the clustering of functional

pathways among bacterial clades using a genomic dataset. This concordance suggests that

functional turnover among bacterial communities from distinct habitats does not only result from

the differential filtering of similar functions among these communities, but also from

phylogenetic correlations among these functions. Functional pathways associated with trait

clusters that were most important for defining functional turnover among bacterial communities

were also those that were the most phylogenetically structured, underlining the important role of

evolutionary history in shaping contemporary distributions of bacteria across ecosystems.

Ecological significance of functional clusters characterizing contrasting habitat types

A few main clusters of correlated functional pathways explained functional variation

among bacterial communities in our metagenomic datasets. The prevalence of genetic

information processing (i.e. replication and repair; translation; folding sorting and degradation)

and nucleotide metabolism in gut bacterial communities can namely be explained by the

importance of DNA repair systems for bacterial colonization of intestines (Vogel-Scheel et al.

2010, Davies et al. 2011). Reactive nitrogen species and oxidative stress indeed play an

important role in host defense against pathogens and have been documented to regulate

microbiome assembly in animal hosts (O'Rourke et al. 2003, Fang and Vasquez-Torres 2020).

A functional cluster composed of pathways related to xenobiotics degradation and

metabolism and to amino acid metabolism were comparatively important in explaining bacterial

community composition in soil and plant microbiota. The prevalence of these metabolic

functions can be linked to the variety of aromatic compounds (i.e. xenobiotics) and amino acids

that plant tissues contain and exude in the soil, which represent a common and varied source of

carbon for terrestrial bacteria (Moe 2013, Pérez-Pantoja et al. 2015). The ability to degrade plant

aromatic compounds was for example linked to a better colonization and growth of bacteria in

rhizosphere habitats (Ledger et al. 2012). Degradation of antibiotic compounds secreted by other

microbes and macro-organisms are also important for survival in the dense and diverse biotic

networks that interact in the soil (Lucas et al. 2019).

Functional pathways linked with the conversion of light to sugar (i.e. photosynthesis and

the TCA cycle), and with the metabolism of cofactors and vitamins were predominant in

bacterial communities found in aquatic habitats, and especially in saltwater, outlining the

importance of autotrophy for bacterial life in aquatic ecosystems. Light-driven proton pumps (i.e.

proteorhodopsins) are for example used by a variety of *Flavobacteriia* to generate energy in

high-light marine environments (Kumagai et al. 2018). The enrichment of these types of

functions in aquatic habitats was contrasted with the enrichment of cell motility pathways in

terrestrial habitats, which could be explained by a more general role for bacterial motility in

tracking nutrients and carbon sources in the soil (i.e. for heterotrophic lifestyles - Turnbull et al.

2001), or in engaging with plants and animals (Akahoshi and Bevins 2022). Finally, the

prevalence of cofactors and vitamin metabolic pathways in saltwater bacteria can be linked to the

documented depletion of several vitamins in oceanic habitats, which has been proposed as an

important filter for planktonic community assembly (Sañudo-Wilhelmy et al. 2012).

Our analyses also identified clusters that explained variation between endophytic and

bacteria and other host-associated bacteria. These clusters both contained functions linked with

community sensing and cell-cell interaction, which likely facilitate establishment on, and

interaction with the host. Functional pathways involved in biofilms were more present in non-

endophytic bacteria, which can be linked to their role in facilitating colonization of host external

and internal surfaces (Bogino et al. 2013, Motta et al. 2021). Quorum sensing functions were

comparatively enriched in endophytic communities where they can contribute to bacterial entry

in the host tissues (Gosai et al. 2020).

The functional classification of bacterial communities portrayed here among habitats is

similar in some ways to those suggested in other studies of microbial functional variation.

Dinsdale et al. (2008) identified functions associated with certain habitats, but focused on broad,

high-level functional categories. Other studies (e.g. Ramírez-Flandes et al. 2019, Malik et al.

2020) found that different genes allowed the functional classification of microbial communities

into different habitat types but focused on a more limited range of habitat types (for example, on

soil microbial communities). By expanding analyses of bacterial functions to identify functional

strategies across metagenomes and genomes without an a priori selection of subsets of functions

or habitats to examine, our analyses revealed that major axes of bacterial trait variation are

structured by clusters of correlated traits that correspond to coherent functional categories across

diverse habitats. It remains an open question whether ecological strategies identified for

macroorganisms such as plants are relevant for understanding the strategies of microorganisms.

In this study, we did not find clear mappings between major groups of correlated traits

explaining variation among bacterial genomes and habitats and the competitor-stress tolerator-

ruderal (CSR) scheme of Grime (1977), whose application to microbial communities has

recently been proposed (Fierer 2017). Our results overall emphasize the role of data-driven

approaches in providing novel insights into the ecological and evolutionary factors that drive

microbial trait variation.

Phylogenetic structure of functional pathways and clusters

Phylogenetically constrained functional clusters based on genomic data were generally

correlated with those obtained from metagenomic data. Concordantly, clusters of functions that

were associated with the first axis of the PCoA and thus explained the most functional variation

among habitats (C2, C9) were also the most phylogenetically 'conserved', having the strongest

signal in the genomic phylogeny across most depths. These results suggest that selection or

constraint (e.g. genetic correlations (Futuyma 2010) or functional barriers to recombination

(Hendrickson et al. 2018)) have been important in shaping the evolution of clusters of correlated

traits in bacteria in a consistent fashion across both clades and habitats, leading to predictable

variation in the ecological strategies of bacteria associated with particular clades. The fact that

we found such clusters despite the likely presence of many transient organisms in environmental

metagenomes provides additional confidence in these results.

Despite identifying three main axes of functional covariation among bacterial traits, other

drivers of functional trait correlations appeared to play a role in structuring trait variation among

ecosystems (Appendix S1: Table S1). First, some metagenomically-derived trait clusters (e.g.

C4, C5, C8) were not observed in the genomic dataset, suggesting that they might result from

environmental filtering on pre-existing trait variation or lateral gene transfer among host-

associated clades rather than a fundamental constraint or trade-off in genome architecture and

evolution. These clusters still showed some level of phylogenetic signal in the genomic

phylogeny, but more so at shallower phylogenetic depths, consistent with filtering of genes

transferred horizontally among related organisms living in a shared habitat (Shapiro et al. 2012).

Second, traits belonging to the cluster C7 did not explain much variation across the axes and

were not enriched in any particular type of high-level functional categories, suggesting they do

not represent part of a functional strategy that has arisen in response to the major axes of

environmental variation encompassed by this dataset. Their lack of correlations in the genomic

dataset as well suggests that they are likely to have been evolving idiosyncratically or to be

subject to more extensive horizontal gene transfer. It is possible that other types of functional

strategies than those identified above are also important for driving within-community niche

partitioning among bacteria, but at the broad scales of the bacterial tree of life and among

metagenomes from distinct habitat types, these types of trade-offs are not important enough to be

consistently identified as clusters of covarying traits. Testing how important the main functional

axes identified in this study are in explaining microbial fitness at smaller spatial and

environmental scales will represent a valuable next research direction for the field.

Conclusion

In conclusion, we used a data-driven functional trait screening approach to identify the

major axes of functional trait covariation in bacterial metagenomes and genomes based on gene

functional annotations. By comparing functional trait clusters in both datasets, we show that the

major strategies driving functional differentiation between metagenomes of co-occurring bacteria

in ecological communities tend to be phylogenetically structured among bacterial genomes. We

also point to the limited overlap between the bacterial ecological strategy axes described here

and the trait classification schemes developed for plants and animals. By reducing the high-

dimensionality of trait variation observed among microorganisms around a small number of

fundamental axes of trait covariation, we make an important step towards generalization of

microbial ecology and of the drivers of biological diversity and bacterial biogeography across

study systems.

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Figure captions

Figure 1. Principal coordinates analysis (PCoa) of bacterial functional pathways across habitats. Communities are represented by alphanumeric symbols where letters designate the habitat (A=Aves, B=Bryophyte, FW=Freshwater, I=Insecta, M=Mammalia, P=Porifera, S=Soil, SW=Saltwater, SWS=Saltwater sediment, VP=Vascular plants) and numbers designate the sample ID within that habitat (see Appendix S1: Table S2). Ellipses are drawn for each habitat and correspond to a 0.95 confidence interval from the group centroid. Functional pathways were fitted onto dimensions 1 and 2 (panel A), and dimensions 3 and 4 (panel C) of the ordination. Only the 20 functional pathways that were the most correlated with each set of dimensions are represented. They are colored by functional cluster (see Figure 2). Taxonomic classes composing these same communities were also fitted onto dimensions 1 and 2 (panel B) and dimensions 3 and 4 (panel D). All taxonomic classes whose relative abundances across samples were significantly correlated with each set of dimensions ($\alpha<0.001$) are represented on the panels.

Figure 2. Hierarchical clustering of bacterial functional pathways across metagenomic samples (panel A) and contribution of functional pathways to the composition of each cluster, per Tier 1 and Tier 2 KEGG category (panel B). Panel A: Each dot at the end of branch represents a functional pathway, for which color codes for Tier 1 KEGG functional category (see legend). Each cluster (labelled C1 to C9) is color-coded to facilitate visual identification in other figures. The standardized explanatory power of each functional pathway across each of the first four dimensions of the metagenomic PCoA is indicated in a heatmap below the clustering. In the heatmap, blue is positively correlated with the dimension, and red is negatively correlated. The relative abundance of each pathway across all samples is also represented. Panel B: Dot size is proportional to the residuals of a chi-square test evaluating how

different from normal is the distribution of functional pathways of a given functional category

among clusters. The more functional pathways of a given category there is in a cluster compared

to the expectation, the bigger and the bluer the dot (see legend). The less functional pathways of

a given category there is in a cluster compared to the expectation, the bigger and the redder the

dot. Color-coding of Tier-2 functional category follows that of panel A.

Figure 3. Tanglegram comparing functional clusters calculated from correlations between

bacterial functional pathways across (left) metagenomic samples from different habitats

and across (right) bacterial genomes. Hierarchical clustering of the genomic data is

phylogenetically constrained (see Methods). Dots at the end of each branch are color-coded by

the Tier-1 KEGG functional category to which the functional pathway belongs. Lines connect

the same functional pathways among datasets and are color-coded by metagenomic cluster.

Figure 4. Phylogenetic signal (Moran's *I*) of functional pathways among clusters and across

phylogenetic depths. Moran's *I* was calculated per pathway using phylogenetic correlograms

across the bacterial genomic phylogeny, from the tip (depth = 0) towards the root of the tree

(depth = 0.0444). Black dots represent the value of estimated marginal means of Moran's I

across the functional pathways of each cluster at each depth. The blue bars are confidence

intervals. The absence of overlap among red arrows of two clusters at a given depth indicates

that their mean phylogenetic signals are significantly different. These pairwise comparisons were

adjusted for multiple testing using the multivariate *t* distribution method (Genz et al. 2021).

Figure 1

Figure 2

Figure 3

Figure 4