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Supporting Online Material for

Diet Drives Convergence in Gut Microbiome Functions Across Mammalian Phylogeny and Within Humans

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This PDF file includes:

Materials and Methods
Figs. S1 to S3
Tables S1 to S11
References (19–34)

Supporting Online Materials

Interactive Plots on the Web

The kinemage files used to generate Figure 1 have been posted on our lab website (19), together with links for panels in that figure. Clicking the links will open java applets that contain an interactive, labeled PCoA plot of the Procrustes analysis used to generate the panel. The viewer can rotate the plot in 3-dimensions, and can also view the first 10 principal coordinate axes. The coloring and naming conventions online are consistent with Figure 1.

Materials and Methods

Subjects

Table S1 lists the mammalian species included in this study and their diet group. Further details about their diets, dietary group classification, plus methods used to recover and store fecal samples have been published previously (1). Eighteen members of the Calorie Restriction Society were recruited for the present study using a procedure approved by the Washington University Human Studies Committee. The volunteers had practiced calorie restriction for an average of 7.6 yrs (range 3.5-21 years). The average age of the study cohort was 59.6 ± 10.6 years and their body mass index was 19.4 ± 1.3 kg/m² (mean \pm S.D.). None of these individuals had consumed antibiotics in the four months prior to enrollment and none had a history of gastrointestinal disorders. Each participant provided a fecal specimen that was frozen at -20°C within 30 min after its production. Samples were maintained at this temperature until they were received (within 24h) at a biospecimen repository where they were anonymized and stored at -80°C prior to metagenomic analyses. The participants kept detailed diet records for four days prior to fecal collection. A dietician analyzed these diet records to quantify macro- and micronutrient content using the Nutrition Data System for Research (NDS-R; version 4.03_31) (16).

Isolation of Fecal DNA and Multiplex Pyrosequencing

All mammalian and human samples were subjected to a common protocol for DNA extraction. Fecal samples were pulverized with a mortar and pestle at -80°C. A 500 mg aliquot of each frozen pulverized sample was re-suspended in a solution containing 500μL of extraction buffer [200mM Tris (pH 8.0), 200mM NaCl, 20mM EDTA], 210μL of 20% SDS, 500μL of phenol:chloroform:isoamyl alcohol (25:24:1) and 500μL of a slurry of 0.1-mm diameter zirconia/silica beads. Cells were then mechanically disrupted using a bead beater (Biospec, maximum setting; 3 min at room temperature), followed by extraction with phenol:chloroform:isoamyl alcohol and precipitation with isopropanol. An aliquot of the DNA was used for PCR amplification and sequencing of bacterial 16S rRNA genes. ~330bp amplicons, spanning variable region 2 (V2) of the gene were generated by using (i) modified primer 8F (5'-
GCCTTGCCAGCCCGCTCAGT**CAGAGTTGATCCTGGCTCAG-3'**) which consists of 454 FLX Amplicon primer B (underlined), a two base linker (bold) and the universal

bacterial primer 8F (italics) and (ii) modified primer 338R (5' GCCTCCCTCGGCCATCAGNNNNNNNNNNNCATGCTGCCCTCCGTAGGAGT 3') which contains 454 FLX Amplicon primer A (underlined), a sample specific, error correcting 12-mer barcode (N's), a two base linker (bold), and the bacterial primer 338R (italics). Three replicate polymerase chain reactions were performed for each fecal DNA sample: each 20-mL reaction contained 100 ng of gel-purified DNA (**Qiaquick**, **QIAGEN**), 8 ml **2.5X HotMaster PCR Mix** (**Eppendorf**) and 0.2 μ M of each primer. PCR conditions consisted of an initial denaturation step performed at 95 °C for 2 min, followed by 30 cycles of denaturation (95°C, 20 s), annealing (52°C, 20 s) and amplification (65°C, 1 min). Amplicons generated from each set of three reactions were subsequently pooled and purified using Ampure magnetic purification beads (Agencourt). The amount of DNA was quantified using Picogreen (Invitrogen), and equimolar amounts of barcoded samples were pooled for each subsequent multiplex 454 FLX pyrosequencer run.

For multiplex shotgun 454 FLX pyrosequencing, each fecal community DNA sample was randomly fragmented by nebulization to 400-800 bp (FLX standard) or 500-800 bp (FLX Titanium), and then labeled with a distinct MID (**Multiplex IDentifier**; Roche) using the MID manufacturer's protocol (general library preparation for FLX standard, Rapid Library preparation for FLX Titanium). Equivalent amounts of up to 12 MID-labeled samples were pooled prior to each pyrosequencer run (454 FLX and Titanium chemistry).

16S rRNA Data Processing and Analysis

16S rRNA amplicon sequences were processed using the QIIME (v1.1) suite of software tools (20); fasta files, quality files and a mapping file indicating the sequence of the 12 nt barcode that corresponded to each sample were used as inputs. QIIME bins pyrosequencer reads by samples according to their barcode, de-noises pyrosequencer data (21), and classifies reads into OTUs on the basis of sequence similarity [e.g., species level phylotypes share $\geq 97\%$ identity (ID)]. QIIME builds a *de novo* taxonomic tree of the sequences based on their similarity and creates a table of samples versus OTUs that can be used, together with the tree, to calculate alpha and beta diversity. Reads were aligned using PyNAST, and chimeric OTUs were removed using the ChimeraSlayer program (22). Procrustes analysis and network analysis were also performed in QIIME, using code made available in release 1.2.0. The network analysis was visualized using Cytoscape v2.6.3 (23), and the nodes and edges were placed using Cytoscape's spring-embedded algorithm.

Taxonomic assignments were made using SILVA-VOTE, an algorithm designed for improved accuracy in taxonomic assignments of V2 16S rRNA reads. Briefly, a non-redundant reference database of 34,181 bacterial 16S rRNA V2 regions was created by clustering the Silva database (release 102). Taxonomic assignment was made at each taxonomic level if more than 75% of the sequences in the cluster had an identical designation at that level (otherwise, the level was designated "unknown"). A representative sequence from each QIIME-identified OTU was compared by BLAST against this custom database, retaining hits with e-value $\leq 10^{-30}$. All BLAST hits within 10% of the best score (up to 100 hits) were used to generate a taxonomic assignment at each taxonomic level. If greater than 50% of the hits shared a designation, this annotation

was assigned to the OTU. Otherwise, the OTU was noted as “nonidentified” at that level. Note that sequences were binned into OTUs with $\geq 97\%$ sequence identity in lane-masked V2 regions. In accord with common convention (24), when 97%ID OTUs had identical taxonomic assignments, they were binned as a single ‘species-level phylotype’. When 97%ID OTUs were assigned to the same bacterial genus, they were treated as a single ‘genus-level phylotype’.

Shotgun Sequencing Data Processing and Annotation

Metagenomic data was annotated using custom Perl scripts. Shotgun reads were filtered using custom Perl scripts and publicly available software to remove (i) all reads less than 60 bases in length, (ii) reads with degenerate bases (N’s), (iii) all duplicates (a known artifact of pyrosequencing), defined as sequences whose initial 20 nucleotides are identical and that share an overall identity of $>97\%$ throughout the length of the shortest read (25) and (iv) in the case of human fecal DNAs from the calorically restricted individuals, all sequences with significant similarity to human reference genomes (BLASTN with e-value $\leq 10^{-5}$, bitscore ≥ 50 , percent identity $\geq 75\%$) to ensure the continued anonymity of samples.

Searches against the KEGG (version 52) and MEROPS (release 9.1) databases were carried out with BLASTX. A sequence read was annotated as the best hit in the database if (i) the E-value was $\leq 10^{-5}$, (ii) the bit score was ≥ 50 , and (iii) the alignment was at least 50% identical between query and subject. In the event that two entries in the database had equivalent BLAST scores as the best hit, the read was annotated with both entries. The KO, E.C., and KEGG Pathways associated with each KEGG sequence were determined using the “ko” file provided by KEGG. Reads were annotated against the CAZy database using procedures described previously (26). For each functional annotation schema, statistical analysis was performed on a matrix containing the count of annotated reads in each sample; e.g., for the KEGG KO data, the matrix contained the list of all possible KOs in the rows of the first column and the sample names in the column headers. The value in each “cell” of the matrix was the number of times that KO was detected as the best BLAST hit of a shotgun read from the sample. All dissimilarity metrics and related calculations were generated with QIIME. For every functional data type, an evenly rarefied matrix of functional assignments was created, a distance matrix using the Bray-Curtis metric was calculated, and results were visualized with Principal Coordinates and Procrustes Analysis.

Taxonomic Composition of Shotgun Sequencing Data

The taxonomic distribution of metagenomic reads was determined using version 3.9 of MEGAN (6). Metagenomic reads were searched against the NCBI non-redundant protein database with BLASTX. We used the additional BLAST parameter $-F "m S"$ as suggested by the authors of the software. The search results were processed in MEGAN with default parameters to generate the taxonomic profile for each sample.

Detecting Differences in E.C. Abundance and Amino Acid Metabolism

E.C. analysis comparing the microbiomes of herbivores and carnivores was implemented with version 1.0.3 of Shotgun Functionalize R (11). As described in the main text, 495 E.C.’s were identified with significantly different relative abundance

between carnivore and herbivore microbiomes, using a Benjamini-Hochberg adjusted p-value of 0.001 as our threshold for significance. These E.C.'s were mapped onto KEGG metabolic pathways and inspected visually. For every amino acid, biosynthetic and degradative reaction pathways were identified using both KEGG annotations and the experimentally confirmed metabolic information collated in the MetaCyc database (27, 28). For every significantly different E.C. that was detected in a major amino acid biosynthetic or catabolic reaction, the relative abundance of the E.C. in the microbiomes was plotted. The objective was to eliminate statistically significant results from the final analysis if there were not a sufficient number of annotations for biological confidence. The following statistically significant E.C.s were not included in the summary analysis: (i) E.C.2.1.4.1 (glycine amidinotransferase) was found in only two microbiomes with low abundance (four total assignments in Bush Dog, two total assignments in Hyena), and (ii) E.C.2.6.1.57 (aromatic amino-acid transferase) which was found in only 7 microbiomes with low abundance (maximum of 15 assignments in Polar Bear, median of two assignments). The results of this analysis are summarized in **Table S6**. For every amino acid, E.C.s detected in biosynthetic or degradative reactions are noted, as well as reversible reactions where likely direction cannot be determined based on available information.

Using Procrustes Analysis to Test whether the Functional Properties of a Microbiome can be Predicted from the Bacterial Species

Procrustes analysis, named after the son of the Greek god Poseidon who fit unsuspecting travelers to a fixed-size bed by stretching them or removing their feet, is a technique for comparing the relative positions of points in two multivariate datasets. The method was first introduced by Hurley and Cattell (7), then generalized by Gower (8), for comparing psychometric datasets. The method has since been employed in macro-ecology (29, 30), and in a recent report used to compare datasets obtained from different regions of the same 16S rRNA sequence (31). We used the implementation of Procrustes analysis in the open-source QIIME microbial community analysis pipeline (20), built using the PyCogent libraries (32) and the Python programming language. As noted in the main text, this procedure yields a measure of fit, M^2 , which is the sum of squared distances between corresponding data points after the transformation. The significance of the association is obtained by a Monte Carlo procedure in which the point labels are randomized, M^2 is recomputed, and the M^2 value of the actual pair of datasets is compared to the empirical distribution of M^2 values observed for the permuted datasets. Because M^2 depends on the sample size and the structure of the data, M^2 values typically cannot be directly compared between datasets, and the statistical significance must be computed for each pair of datasets separately. In these studies, we used 1,000 replicates for calculating P-values.

We applied the Bray-Curtis distance metric to the functional data to obtain PCoA coordinates for comparison with the 16S rRNA data. We also tested additional qualitative (Jaccard) and quantitative (Canberra, Gower) distance metrics on the KO data. All of these distance metrics led to the same essential conclusion; the agreement between the weighted UniFrac distances and the KO distance matrix was significant over the first three dimensions of the Procrustes plot ($p<0.05$).

Assessing Clustering Using the Monte Carlo Procedure

Monte Carlo simulations allow researchers to directly determine the probability of obtaining a result more extreme than the observed metric with random sampling of the data. Importantly, this test makes no *a priori* assumptions of the underlying structure of the data (e.g., a parametric distribution) and can thus be powerfully deployed with a range of experimental results. To assess sample clustering by diet, we computed the t-statistic of the UniFrac distances of 16S rRNA data and (separately) the Bray-Curtis distances of KO data, comparing the average distance between the fecal bacterial communities of herbivores to the average distance between carnivore communities. This t-statistic was treated as the “observed” result. Then, the pairwise distance matrix was randomly permuted a set number of times by shuffling the sample labels; in this study, we always performed 1,000 independent permutations. For each permutation, the t-statistic was recalculated using the new permuted labels. The distribution of the t-test statistic for the permutations was compared to the “observed” metric from the real data. The fraction of times a permutation resulted in a metric more extreme than the observed metric is the p-value, the probability that the observed result could have arisen by chance from the underlying data. For example, if 11 of the 1,000 random permutations comparing the average UniFrac distance of herbivore fecal bacterial communities to the average UniFrac distance of carnivore communities had a t-statistic more extreme than the actual observed statistic, the reported p-value would be 11/1000, or 0.011. All Monte Carlo simulations were implemented using QIIME scripts. The same procedure was employed to assess the clustering of conspecific samples, here comparing unweighted and weighted UniFrac distances between animals of the same species to distances between animals of different species.

Regression Analysis of Data Obtained from Human Subjects

Linear regression was carried out using the R statistical software package with a simple linear model (33). Principal Coordinates were generated using QIIME as described previously for the 16S rRNA OTU data and functional KO data using the Bray-Curtis distance metric, and for the 16S rRNA data using the weighted and unweighted UniFrac distances. Principal Coordinates for the other types of functional annotation were calculated but not included in linear regression model because they had a high Pearson correlation coefficient with the KO Principal Coordinates and thus did not represent independent response variables (E.C correlation=0.97, CAZyme correlation=0.87, peptidase correlation=0.73).

For each individual, the average daily intake of carbohydrates, proteins, and insoluble fiber was calculated based on the dietary records from the four days proceeding fecal sample donation. The position for each calorie-restricted individual’s gut bacterial community along Principal Coordinate 1 was regressed against each of the three dietary components using a simple linear model. The p-value resulting from the analysis was multiplied by three to adjust for the multiple hypotheses tested (Bonferroni adjustment). We also implemented a multiple linear regression using all three dietary components and their interactions as explanatory variables, with the Principal Coordinate positions as the response variable. We used backwards stepwise selection to remove non-significant terms from the model. In no case did a mixed model generate significant interactions beyond the simple linear models tested previously.

The average daily calories consumed by our cohort ranged from 1207-2551 kCal/day (mean 1673, standard deviation 395 kCal/day). We regressed total kilocalories consumed against the PC1 coordinate of our samples for all data categories (16S rRNA and functional data). After correcting for multiple-hypothesis testing, total calories were not significantly associated with any of these categories ($p > 0.05$).

Results

Prediction of Community Functional Profiles from Species Assemblage Data using a Nearest-Neighbor Model

As noted in the main text, the strong correlation between bacterial 16S rRNA and functional profiles made us wonder if the functional configuration of a microbiome could be predicted from its 16S rRNA sequences. To test this idea, we developed a nearest-neighbor model. For a given sample, we predicted its functional composition to be the same as that sample's nearest neighbor (using the weighted UniFrac distance comparison of 16S rRNA data). To assess the quality and significance of these predictions, we compared the average root mean squared error (RMSE) of our model to the average RMSE for one million Monte Carlo trials where each sample's nearest neighbor was chosen at random from the remaining samples. The UniFrac nearest neighbor generated a significantly better functional prediction than a random neighbor for all four types of functional; for KOs, E.C.s, peptidases, and CAZymes, no permutation in the one million trials had a lower RMSE than the UniFrac prediction ($p=0$). Using the unweighted UniFrac distances also led to predicted functional profiles that were significantly better than would be expected by chance (KOs, $p=0$; E.C.s, $p=0$; proteases, $p=0.000252$; CAZymes, $p=0$).

Phylogenetic Congruence Testing

We examined the congruence between host phylogeny and the presence of bacterial taxa or particular enzymatic (or other protein) functions in host microbiomes by comparing host phylogeny to both the OTUs and KOs present in the fecal samples. We first generated a table of each OTU's or KO's presence or absence in each sample, and selected a number of sequences (without replacement) from each sample's sequences to avoid biases associated with uneven sequencing effort among samples (we selected 1,000 bacterial 16S rRNA sequences from each sample for the OTU analysis, and 5,000 assigned shotgun sequences for the KO analysis). We then eliminated OTUs or KOs present in only one sample, as they were not useful for assessing the congruence between fecal microbiomes and host phylogeny. We then searched for OTUs or KOs that were present in all members of any distinct monophyletic lineage and absent in all other samples included in this study. We compared this result to the results obtained on a randomized host phylogeny where the samples we obtained were associated with randomly chosen tips of the host phylogeny. To summarize the results, we grouped OTUs with assignments to identical bacterial genera, and looked for bacterial genera enriched for OTUs matching the mammalian phylogeny. Of the 198 different genera assignments detected in the subsampled OTU dataset, three (Prevotella, Barnesiella, and Bacteroides) were found to be significantly enriched in matching OTUs (see main text; $p < 0.05$ relative

to a binomial distribution using the overall abundance of matching OTUs). OTUs that lacked a genera assignment were not reported when identifying bacterial genera congruent with mammalian phylogeny. Of the 668 OTUs not assigned to a named genera, 29 (4%) matched a monophyletic group in the mammalian tree, a similar proportion found in the genus-assigned OTUs.

To account for OTUs and functions whose presence/absence pattern did not exactly match a monophyletic group of mammals, we performed a test for increased presence within a monophyletic group relative to the expected value given the overall abundance of the OTU or function over all samples, using a binomial distribution. Categorizing these OTUs into genera as before, Prevotella, Barnesiella, and Bacteroides were significantly enriched for OTUs whose presence was congruent with the mammalian phylogeny, as well as Ureaplasma, Paludibacter and Pedobacter. Employing this method, we found 90 KOs, 2 CAZymes (of 119 tested), and 1 (of 274 tested) proteases congruent with host phylogeny.

Additionally, we employed the method of Ochman *et. al.* (34) as another test for co-phylogeny. Their study examined four wild primate species and their fecal microbiota, and created a maximum parsimony tree of the gut communities using a character matrix of bacterial species normalized abundances that was compared to the primate phylogeny (as determined by mitochondrial DNA sequence). To implement their approach, we used MESQUITE rather than PAUP for parsimony inference and eliminated bacterial taxa that appeared in only a single sample (non-parsimony-informative sites). We also tried a variety of parsimony variants (ordered states based on discretized z-scores, with SPR and NNI heuristics for tree search). We were able to reproduce their results for the four primate species using their source data. However, when applied to our larger mammalian tree of 33 species, we did not see an overall match between the host tree and the parsimony tree inferred from fecal bacterial communities, providing further evidence supporting our conclusion that the distribution of bacterial species does not mirror host phylogeny over the whole of the mammalian tree. We did not sample any one closely related clade with sufficient density to perform extensive tests of whether the bacterial community signal Ochman and coworkers identified dissipates once a particular evolutionary depth is reached.

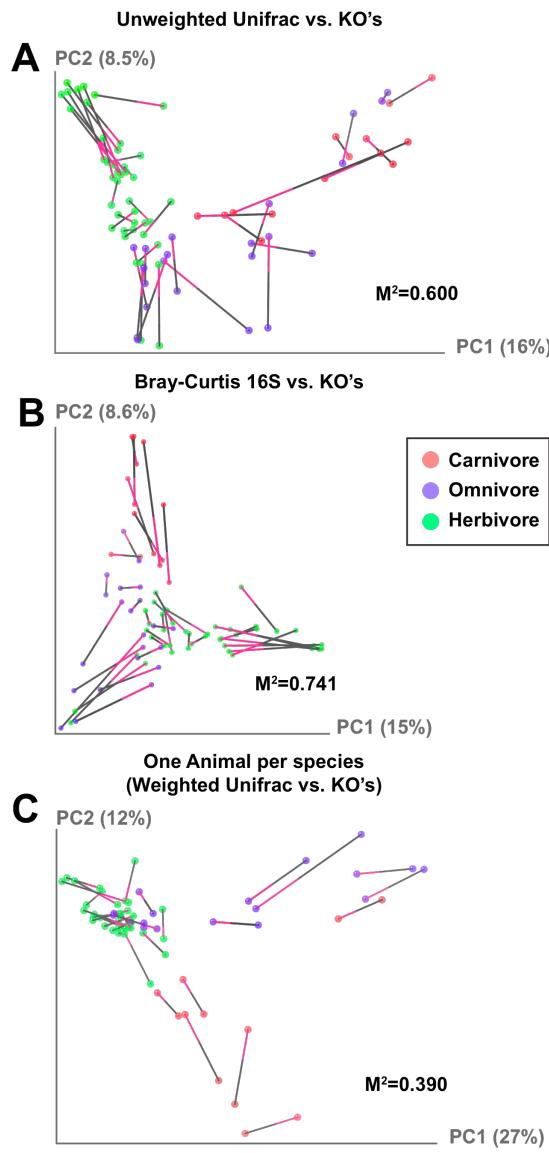


Fig. S1.

Procrustes analysis is robust to a variety of computational approaches. Procrustes analysis of 16S rRNA sequences (weighted UniFrac, unweighted UniFrac, OTU counts) against KO annotation of shotgun pyrosequencing reads. Every sphere represents a single mammalian fecal community and is colored by host diet. The black end of each line connects to the 16S data for the sample, while the orange end is connected to the functional annotation data. The fit of each Procrustes transformation over the first three dimensions, is reported as the M^2 value. (A) Procrustes analysis of 16S rRNA data (unweighted UniFrac) against KEGG Orthology (KO) groups. (B) Procrustes analysis of OTU counts (Bray Curtis metric) against KOs. (C) Procrustes analysis of 16S rRNA data (weighted UniFrac) against KOs, using only one animal sample from each of the 33 mammalian species.

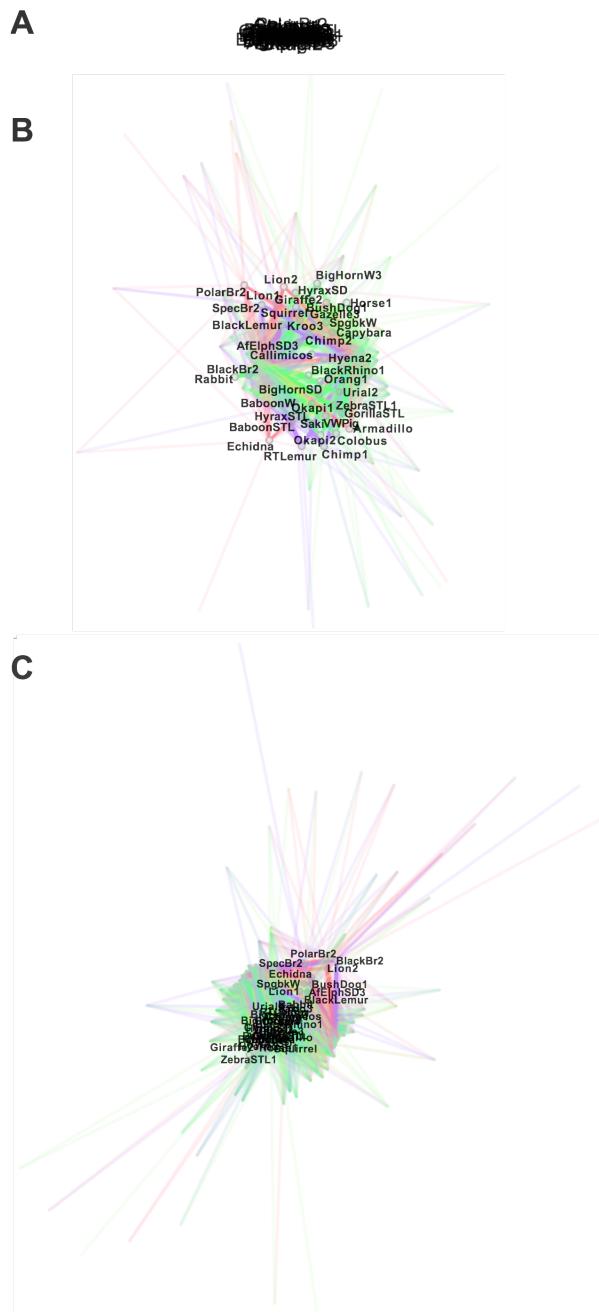


Fig. S2.

Bipartite network analysis. **(A)** Close-up of animal node labels for KO bipartite graph from **Fig. 2B** in main text. **(B-C)** Bipartite network diagrams of evenly sampled CAZymes [glycoside hydrolases] (**B**) or peptidases (**C**). Labeled circles (nodes) denote animal hosts, and are colored by host diet. Lines (edges) radiating from the host nodes connect to microbiome gene nodes representing a single glycoside hydrolase or peptidase found in the host fecal microbiome.

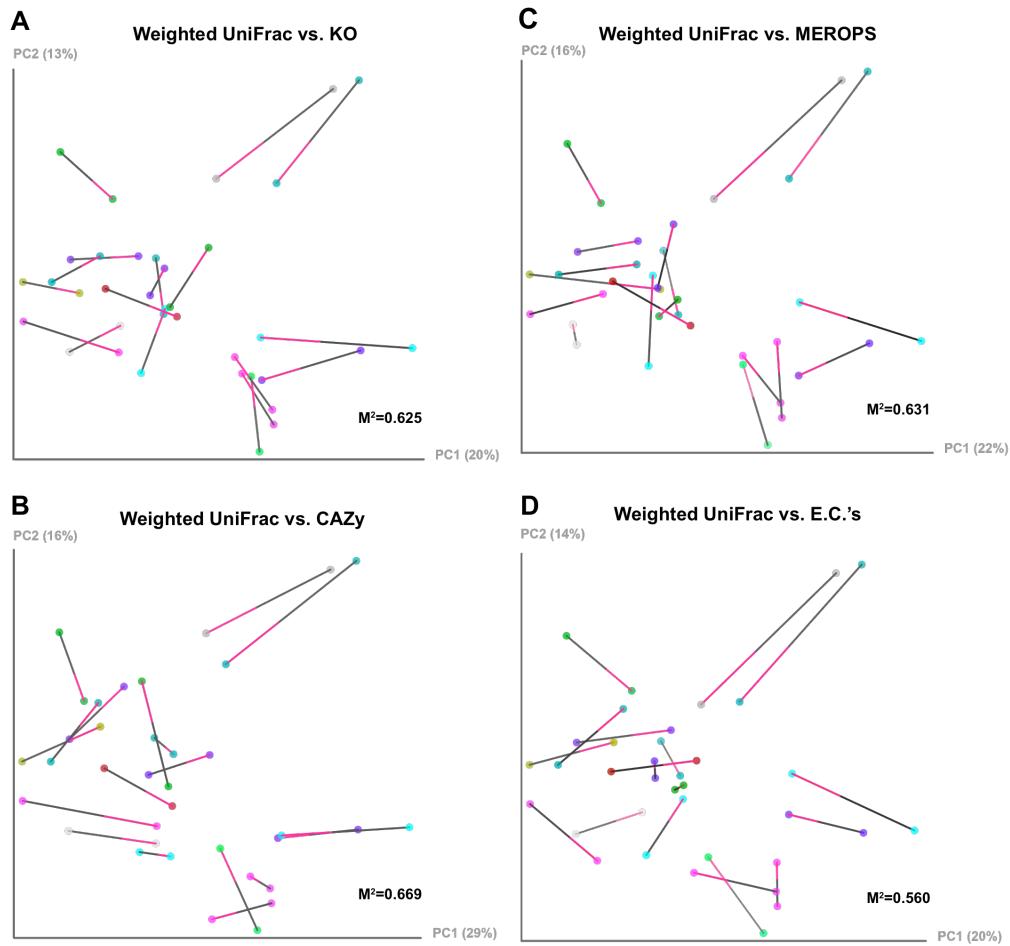


Fig. S3.

Procrustes analysis shows that the bacterial lineages and microbiome gene content from humans who practice caloric restriction with adequate nutrition give similar clustering patterns. (A-D) Procrustes analysis of bacterial 16S rRNA sequences (weighted UniFrac) against KOs, CAZymes (glycoside hydrolases), MEROPS (peptidases), and Enzyme Commission numbers (E.C.s). Every sphere represents a single mammalian fecal community and is colored by host diet. The black end of each line connects to the 16S data for the sample, while the orange end is connected to the functional annotation data. The fit of each Procrustes transformation over the first three dimensions is reported as the M^2 value. Spheres are colored differently for each human host.

SampleID	Common Name	Provenance	Diet	Gut Phys-	Order	Family	Genus / species
AfElphSD3	African Elephant	SD	H	HG	Proboscidae	Elephantidae	<i>Loxodonta africana</i>
Armadillo	Southern Three-banded Armadillo	STL	C	S	Xenarthra	Dasypodidae	<i>Tolypeutes matacus</i>
BaboonSTL	Hamadryas Baboon	STL	O	S	Primates	Cercopithecidae	<i>Papio hamadryas</i>
BaboonW	Hamadryas Baboon	W	O	S	Primates	Cercopithecidae	<i>Papio hamadryas</i>
BigHornSD	Bighorn Sheep	SD	H	FG	Artiodactyla	Bovidae	<i>Ovis canadensis</i>
BigHornW3	Bighorn sheep	W	H	FG	Artiodactyla	Bovidae	<i>Ovis canadensis</i>
BlackBr2	North American Black Bear	STL	O	S	Carnivora	Ursidae	<i>Ursus americanus</i>
BlackLemur	Black Lemur	STL	O	S	Primates	Lemuridae	<i>Eulemur macaco macaco</i>
BlackRhino1	Black Rhinoceros	STL	H	HG	Perissodactyla	Rhinocerotidae	<i>Diceros bicornis</i>
BushDog1	Bush Dog	STL	C	S	Carnivora	Canidae	<i>Speothos venaticus</i>
Callimicos	Goeldi's Marmoset	STL	O	S	Primates	Cebidae	<i>Callimico goeldii</i>
Capybara	Capybara	STL	H	HG	Rodentia	Caviidae	<i>Hydrochaeris hydrochaeris</i>
Chimp1	Chimpanzee	STL	O	S	Primates	Hominidae	<i>Pan troglodytes</i>
Chimp2	Chimpanzee	STL	O	S	Primates	Hominidae	<i>Pan troglodytes</i>
Colobus	Black and White Colobus Monkey	STL	H	FG	Primates	Cercopithecidae	<i>Colobus guereza</i>
Echidna	Short-beaked Echidna	STL	C	S	Monotremata	Tachyglossidae	<i>Tachyglossidae aculeatus</i>
Gazelle3	Speke's Gazelle	STL	H	FG	Artiodactyla	Bovidae	<i>Gazella spekei</i>
Giraffe2	Reticulated Giraffe	STL	H	FG	Artiodactyla	Giraffidae	<i>Giraffa camelopardalis reticulata</i>
GorillaSTL	Western Lowland Gorilla	STL	H	HG	Primates	Hominidae	<i>Gorilla gorilla</i>
Horse1	Horse	W	H	HG	Perissodactyla	Equidae	<i>Equus equus</i>
Hyena2	Spotted Hyena	STL	C	S	Carnivora	Hyaenidae	<i>Crocuta crocuta</i>
HyraxSD	Rock Hyrax	SD	H	FG	Hyracoidae	Procaviidae	<i>Procavia capensis</i>
HyraxSTL	Rock Hyrax	STL	H	FG	Hyracoidae	Procaviidae	<i>Procavia capensis</i>
Kroo3	Red Kangaroo	STL	H	FG	Diprotodontia	Macropidae	<i>Macropus rufus</i>
Lion1	Lion	STL	C	S	Carnivora	Pantherinae	<i>Panthera leo</i>
Lion2	Lion	STL	C	S	Carnivora	Pantherinae	<i>Panthera leo</i>
Okapi1	Okapi	STL	H	FG	Artiodactyla	Giraffidae	<i>Okapia johnstoni</i>
Okapi2	Okapi	STL	H	FG	Artiodactyla	Giraffidae	<i>Okapia johnstoni</i>
Orang1	Sumatran Orangutan	STL	H	HG	Primates	Hominidae	<i>Pongo pygmaeus abelii</i>
PolarBr2	Polar Bear	STL	C	S	Carnivora	Ursidae	<i>Ursus maritimus</i>
Rabbit	European Rabbit	STL	H	HG	Lagomorpha	Leporidae	<i>Oryctolagus cuniculus</i>
RTLemur	Ring-tailed Lemur	STL	O	S	Primates	Lemuridae	<i>Lemur catta</i>
Saki	White-faced Saki	STL	O	S	Primates	Pitheciidae	<i>Pithecia pithecia</i>
SpecBr2	Spectacled Bear	STL	O	S	Carnivora	Ursidae	<i>Tremarctos ornatus</i>
SpgbkW	Springbok	W	H	FG	Artiodactyla	Bovidae	<i>Antidorcas marsupialis</i>
Squirrel	Prevost's Squirrel	STL	O	S	Rodent	Sciuridae	<i>Callosciurus prevostii</i>
Urial2	Transcaspian Urial	STL	H	FG	Artiodactyla	Bovidae	<i>Ovis orientalis arkal</i>
VWPig	Visayam Warty Pig	SD	H	FG	Artiodactyla	Suidae	<i>Sus cebifrons</i>
ZebraSTL1	Grevy's Zebra	STL	H	HG	Perissodactyla	Equidae	<i>Equus grevyi</i>

Table S1.

Metadata on 39 non-human mammals included in this study, including provenance, diet, gut physiology, and phylogenetic order.

SampleID	Total 16S rRNA V2 reads (De-noised, Chimera Checked)	Total OTUs (97% ID)	Shannon's Index*
AfElphSD3	5130	895	7.88
Armadillo	4889	380	6.06
BaboonSTL	4480	451	5.64
BaboonW	2788	396	6.28
BigHornSD	4592	1003	8.08
BigHornW3	2281	497	4.80
BlackBr2	2404	30	0.93
BlackLemur	2997	151	3.65
BlackRhino1	3781	713	7.19
BushDog1	3345	137	4.22
Callimicos	4549	137	3.56
Capybara	3232	661	7.49
Chimp1	3477	564	7.24
Chimp2	3814	553	7.04
Colobus	1979	430	7.24
Echidna	3151	254	5.45
Gazelle3	4296	902	7.99
Giraffe2	3220	701	7.53
GorillaSTL	3317	515	7.06
Horse1	3982	1269	8.77
Hyena2	1900	235	6.13
HyraxSD	3431	504	7.10
HyraxSTL	3485	428	6.11
Kroo3	4854	870	7.93
Lion1	3692	110	3.62
Lion2	4026	141	4.05
Okapi1	4628	929	8.07
Okapi2	4838	943	8.01
Orang1	3299	543	7.12
PolarBr2	3303	84	3.23
Rabbit	3703	301	5.49
RTLemur	4439	571	7.19
Saki	7359	325	5.96
SpecBr2	3143	33	2.01
SpgbkW	4341	860	7.69
Squirrel	2923	189	2.39
Urial2	6281	1243	8.24
VWPig	3750	690	7.18
ZebraSTL1	4576	1083	7.95

* average of 50 iterations at 1500 reads per sample

Table S2.
Mammal 16S rRNA sequencing statistics

SampleID	Sequencing chemistry	Total shotgun reads (quality filtered, dereplicated)	Mean read length (\pm standard deviation) [nt]	Number of reads with KEGG Orthology group (KO) assignment	Total E.C. assignments	Number of reads with MEROPS assignment	Number of reads with CAZy assignment*
AfElphSD3	FLX	140869	170 (\pm 66)	24624	17065	1553	1664
Armadillo	FLX	28737	226 (\pm 46)	9827	6437	685	641
BaboonSTL	FLX	52293	224 (\pm 39)	14805	9862	1104	821
BaboonW	FLX	31429	202 (\pm 60)	8039	5184	481	395
BigHornSD	Titanium	45680	399 (\pm 124)	14302	9636	1154	1145
BigHornW3	Titanium	51875	407 (\pm 122)	16006	10600	1168	1346
BlackBr2	Titanium	68884	410 (\pm 123)	44308	27396	2761	1641
BlackLemur	FLX	37116	210 (\pm 50)	13510	9237	966	1037
BlackRhino1	FLX	94535	203 (\pm 59)	22859	15729	1791	1762
BushDog1	FLX	64646	223 (\pm 48)	23063	14893	1584	1281
Callimicos	FLX	51502	217 (\pm 41)	16491	11480	1297	1122
Capybara	FLX	107461	220 (\pm 46)	28456	19755	2025	2588
Chimp1	FLX	65969	222 (\pm 42)	19179	12829	1336	1091
Chimp2	Titanium	19568	322 (\pm 159)	5142	3297	365	296
Colobus	FLX	67621	218 (\pm 47)	19175	13037	1387	1370
Echidna	FLX	65266	231 (\pm 43)	28973	18621	1775	822
Gazelle3	FLX	68262	221 (\pm 44)	18741	12737	1352	1342
Giraffe2	FLX	53668	182 (\pm 62)	13543	9134	967	1043
GorillaSTL	FLX	24933	154 (\pm 65)	5397	3622	376	294
Horse1	FLX	98800	213 (\pm 47)	23835	16451	1628	2092
Hyena2	FLX	68456	228 (\pm 42)	22739	14679	1501	1187
HyraxSD	FLX	42140	217 (\pm 45)	12362	8291	934	804
HyraxSTL	FLX	66102	228 (\pm 39)	17711	11951	1285	1575
Kroo3	FLX	22049	220 (\pm 47)	6227	4202	376	376
Lion1	FLX	14236	221 (\pm 49)	5721	3677	346	305
Lion2	Titanium	75012	413 (\pm 120)	36205	22217	2683	2035
Okapi1	FLX	32804	208 (\pm 53)	9183	6248	649	683
Okapi2	FLX	22615	223 (\pm 43)	6373	4234	425	463
Orang1	FLX	29219	203 (\pm 57)	7940	5235	521	326
PolarBr2	Titanium	56380	413 (\pm 122)	35745	21472	2414	958
Rabbit	FLX	69106	232 (\pm 42)	18933	12576	1210	1412
RTLemur	Titanium	50463	409 (\pm 119)	16659	10904	1235	1789
Saki	Titanium	127069	411 (\pm 119)	37487	25394	3238	4078
SpecBr2	Titanium	38999	386 (\pm 133)	19407	12982	1266	1071
SpgbkW	FLX	48935	232 (\pm 39)	15946	10697	1148	902
Squirrel	FLX	43724	231 (\pm 42)	16070	10925	1075	1265
Urial2	FLX	31996	199 (\pm 59)	7858	5392	586	531
VWPig	Titanium	59174	392 (\pm 127)	18638	12624	1456	1923
ZebraSTL1	FLX	25693	224 (\pm 46)	6198	4148	418	512

*All reads assigned as Glycoside Hydrolase, Polysaccharide Lyase, or Carbohydrate Esterase

Table S3.

Mammal fecal community DNA shotgun pyrosequencing datasets: statistics.

Table S4A

SampleID	Total number of reads with hit in nr (%)	Total number of reads with no hit (%)
AfElphSD3	94105 (66.8%)	46764 (33.2%)
Armadillo	26074 (90.7%)	2663 (9.3%)
BaboonSTL	45503 (87.0%)	6790 (13.0%)
BaboonW	25559 (81.3%)	5870 (18.7%)
BigHornSD	41760 (91.4%)	3920 (8.6 %)
BigHornW3	47427 (91.4%)	4448 (8.6%)
BlackBr2	66547 (96.6%)	2337 (3.4%)
BlackLemur	33617 (90.6%)	3499 (9.4%)
BlackRhino1	76007 (80.4%)	18528 (19.6%)
BushDog1	59481 (92.0%)	5165 (8.0 %)
Callimicos	45531 (88.4%)	5971 (11.6%)
Capybara	91644 (85.3%)	15817 (14.7 %)
Chimp1	56975 (86.4%)	8994 (13.6%)
Chimp2	15646 (80.0%)	3922 (20.0%)
Colobus	58295 (86.2%)	9326 (13.8%)
Echidna	58111 (89.0%)	7155 (11.0%)
Gazelle3	57696 (84.5%)	10566 (15.5 %)
Giraffe2	42041 (78.3%)	11627 (21.7 %)
GorillaSTL	18249 (73.2%)	6684 (26.8%)
Horse1	81167 (82.2%)	17633 (17.8%)
Hyena2	61471 (89.8%)	6985 (10.2%)
HyraxSD	36955 (87.7%)	5185 (12.3%)
HyraxSTL	57433 (86.9%)	8669 (13.1%)
Kroo3	18676 (84.7%)	3373 (15.3%)
Lion1	13248 (93.1%)	988 (6.9%)
Lion2	71512 (95.3%)	3500 (4.7%)
Okapi1	27257 (83.1%)	5547 (16.9%)
Okapi2	19134 (84.6%)	3481 (15.4%)
Orang1	24438 (83.6%)	4781 (16.4%)
PolarBr2	53835 (95.2%)	2545 (4.5%)
Rabbit	60272 (87.2%)	8834 (12.8%)
RTLemur	47200 (93.5%)	3263 (6.5%)
Saki	115967 (91.3%)	11102 (8.7%)
SpecBr2	35980 (92.3%)	3019 (7.7%)
SpgbkW	41979 (85.8%)	6956 (14.2%)
Squirrel	40193 (91.9%)	3531 (8.1%)
Urial2	24902 (77.8%)	7094 (22.2%)
VWPig	54002 (91.3%)	5172 (8.7%)
ZebraSTL1	21268 (82.8%)	4425 (17.2%)

Table S4B

SampleID	Bacteria	Eukarya	Archaea	Viruses	Cellular Organism / Root	Not assigned
AfElphSD3	73.54%	4.52%	1.141%	0.006%	17.59%	3.21%
Armadillo	89.82%	1.03%	ND	ND	4.84%	4.31%
BaboonSTL	77.90%	3.66%	0.149%	0.022%	13.73%	4.54%
BaboonW	80.05%	2.18%	0.184%	ND	11.90%	5.69%
BigHornSD	78.23%	2.23%	0.259%	ND	15.33%	3.96%
BigHornW3	77.72%	2.71%	0.346%	0.036%	15.47%	3.72%
BlackBr2	95.15%	0.15%	0.017%	0.089%	3.04%	1.55%
BlackLemur	89.33%	0.99%	0.027%	0.036%	6.14%	3.48%
BlackRhino1	78.12%	3.74%	0.345%	0.007%	14.58%	3.21%
BushDog1	91.22%	1.15%	ND	0.000%	5.12%	2.51%
Callimicos	88.54%	1.77%	ND	0.011%	6.48%	3.19%
Capybara	77.75%	3.72%	1.164%	0.010%	14.30%	3.06%
Chimp1	83.46%	2.78%	1.773%	ND	8.54%	3.45%
Chimp2	78.03%	1.99%	0.217%	ND	13.50%	6.27%
Colobus	78.29%	3.90%	0.379%	0.026%	13.62%	3.79%
Echidna	87.79%	1.29%	ND	0.083%	7.19%	3.66%
Gazelle3	79.00%	3.81%	0.802%	0.009%	12.31%	4.07%
Giraffe2	77.57%	3.17%	0.480%	ND	14.34%	4.45%
GorillaSTL	79.70%	1.38%	1.255%	ND	11.57%	6.10%
Horse1	71.02%	5.35%	0.463%	0.007%	19.77%	3.39%
Hyena2	85.01%	2.35%	0.011%	ND	9.16%	3.46%
HyraxSD	79.75%	2.51%	0.674%	ND	12.52%	4.55%
HyraxSTL	80.23%	3.17%	0.919%	ND	12.09%	3.59%
Kroo3	77.37%	2.66%	ND	ND	12.47%	7.51%
Lion1	88.90%	0.52%	ND	0.038%	4.97%	5.56%
Lion2	92.14%	1.05%	ND	0.063%	4.67%	2.07%
Okapi1	79.70%	2.65%	0.352%	ND	11.90%	5.40%
Okapi2	77.40%	2.32%	0.930%	ND	12.09%	7.27%
Orang1	78.86%	2.12%	0.982%	ND	12.44%	5.61%
PolarBr2	91.36%	0.38%	0.000%	0.305%	5.44%	2.52%
Rabbit	78.92%	3.58%	0.037%	ND	13.76%	3.70%
RTLemur	85.26%	1.40%	0.030%	ND	10.47%	2.85%
Saki	83.57%	4.83%	0.197%	0.015%	9.26%	2.12%
SpecBr2	88.51%	0.86%	0.019%	2.118%	5.53%	2.97%
SpgbkW	80.45%	3.00%	0.195%	ND	11.64%	4.72%
Squirrel	90.64%	1.01%	0.030%	ND	5.21%	3.11%
Urial2	78.05%	2.71%	1.426%	ND	11.60%	6.22%
VWPig	80.94%	2.36%	0.200%	ND	13.35%	3.15%
ZebraSTL1	69.46%	4.03%	0.244%	ND	18.53%	7.73%

"ND" = No reads detected in this taxonomic branch

Table S4.

Mammal fecal community DNA shotgun pyrosequencing datasets: phylogenetic assignments. (A) Summary of total hits against the NCBI non-redundant (nr) database. (B) Percentage of all reads assigned to the major phylogenetic divisions (normalized to total number of reads with hit in NCBI nr database).

E.C. Number	Coef-ficient*	AIC [†]	P-value	Adjusted P-value (BH) [‡]	Annotation [§]
EC2.7.1.69	1.644	596.7	7.8e-320	1.3e-316	Protein-N(pi)-phosphohistidine--sugar phosphotransferase
EC2.7.13.3	1.027	477.0	1.0E-136	8.0E-134	Histidine kinase
EC3.6.3.12	2.843	319.8	4.4E-112	2.3E-109	Potassium-transporting ATPase
EC1.7.99.4	2.494	319.1	1.5E-69	6.2E-67	Nitrate reductase
EC3.2.1.86	1.767	341.0	2.0E-66	6.4E-64	6-phospho-beta-glucosidase
EC1.2.7.3	-1.348	235.5	1.7E-56	4.6E-54	2-oxoglutarate synthase
EC1.---.	0.656	439.8	7.9E-55	1.8E-52	NA
EC4.1.1.31	3.032	139.0	6.3E-45	1.3E-42	Phosphoenolpyruvate carboxylase
EC2.7.9.1	-0.991	310.8	1.0E-44	1.8E-42	Pyruvate, phosphate dikinase
EC2.4.1.12	3.341	187.9	1.3E-43	2.0E-41	Cellulose synthase (UDP-forming)
EC1.17.4.1	1.045	210.2	1.3E-37	2.0E-35	Ribonucleoside-diphosphate reductase
EC1.2.7.8	-1.278	250.8	2.5E-37	3.4E-35	Indolepyruvate ferredoxin oxidoreductase
EC1.6.5.-	-1.487	241.6	5.6E-37	6.9E-35	NA
EC3.1.21.3	-0.688	359.5	7.2E-37	8.2E-35	Type I site-specific deoxyribonuclease
EC1.12.99.6	2.596	161.2	1.3E-36	1.4E-34	Hydrogenase (acceptor)
EC1.10.3.-	1.441	237.6	6.4E-36	6.5E-34	NA
EC3.4.11.2	2.140	177.4	4.5E-35	4.3E-33	Membrane alanyl aminopeptidase
EC2.1.1.72	-0.654	383.4	1.9E-33	1.7E-31	Site-specific DNA-methyltransferase (adenine-specific)
EC1.14.13.-	3.101	160.2	4.3E-33	3.7E-31	NA
EC4.1.1.3	-0.880	306.6	1.4E-32	1.1E-30	Oxaloacetate decarboxylase
EC2.1.1.14	2.066	157.0	1.6E-31	1.2E-29	5-methyltetrahydropteroylglutamate--homocysteine S-methyltransferase
EC3.6.3.2	2.098	144.3	5.5E-31	4.0E-29	Magnesium-importing ATPase
EC3.2.1.21	-0.615	305.8	1.8E-30	1.3E-28	Beta-glucosidase
EC2.7.3.9	1.075	176.2	5.1E-30	3.4E-28	Phosphoenolpyruvate--protein phosphotransferase
EC4.1.1.17	2.830	134.2	1.3E-29	8.6E-28	Ornithine decarboxylase
EC2.7.7.42	3.556	132.4	1.0E-28	6.4E-27	[Glutamate--ammonia-ligase] adenylyltransferase
EC1.1.1.1	0.708	242.7	1.4E-28	8.2E-27	Alcohol dehydrogenase
EC5.1.2.3	2.648	202.7	4.4E-28	2.5E-26	3-hydroxybutyryl-CoA epimerase
EC1.2.7.-	-0.586	280.5	2.7E-27	1.5E-25	NA
EC6.4.1.2	1.039	190.7	5.6E-27	3.0E-25	Acetyl-CoA carboxylase
EC1.1.1.49	2.907	127.2	2.5E-26	1.3E-24	Glucose-6-phosphate dehydrogenase
EC4.2.1.20	-0.974	202.6	3.4E-26	1.7E-24	Tryptophan synthase
EC1.2.1.2	0.975	293.4	1.4E-25	6.8E-24	Formate dehydrogenase
EC1.8.1.2	2.504	154.5	3.1E-25	1.5E-23	Sulfite reductase (NADPH)
EC3.1.3.-	1.659	226.7	7.6E-25	3.5E-23	NA
EC2.3.3.9	2.746	135.8	3.4E-24	1.5E-22	Malate synthase
EC2.1.1.52	1.617	172.6	4.2E-24	1.8E-22	rRNA (guanine-N(2)-)methyltransferase
EC1.7.2.3	2.274	177.0	5.6E-24	2.4E-22	Trimethylamine-N-oxide reductase (cytochrome c)
EC2.7.8.20	3.610	91.6	3.1E-23	1.3E-21	glycerophosphotransferase
EC3.1.11.-	2.802	139.9	3.3E-23	1.3E-21	NA
EC3.4.24.55	4.588	92.6	6.4E-23	2.5E-21	Pitrilysin
EC3.2.1.17	2.422	132.2	1.1E-22	4.1E-21	Lysozyme
EC1.8.1.7	2.530	133.7	2.5E-22	9.5E-21	Glutathione-disulfide reductase
EC1.2.4.2	2.407	169.6	2.8E-22	1.0E-20	Oxoglutarate dehydrogenase (succinyl-transferring)
EC2.3.1.15	3.136	121.9	3.8E-22	1.3E-20	Glycerol-3-phosphate O-acyltransferase
EC3.1.26.12	2.348	137.0	4.6E-22	1.6E-20	Ribonuclease E
EC1.1.5.2	20.587	65.8	6.5E-22	2.2E-20	Quinoprotein glucose dehydrogenase
EC2.4.1.20	-1.433	188.2	2.1E-21	7.0E-20	Cellobiose phosphorylase
EC1.6.99.3	1.407	185.5	4.4E-21	1.5E-19	NADH dehydrogenase
EC1.17.1.4	0.940	201.5	1.4E-20	4.4E-19	Xanthine dehydrogenase
EC2.4.1.8	2.530	170.3	1.9E-20	6.1E-19	Maltose phosphorylase
EC4.2.1.17	1.321	290.8	2.7E-20	8.2E-19	Enoyl-CoA hydratase
EC1.21.4.1	1.978	124.7	4.1E-20	1.2E-18	D-proline reductase (dithiol)
EC1.2.3.3	3.446	148.2	1.8E-19	5.3E-18	Pyruvate oxidase
EC3.3.1.1	-1.766	142.0	3.0E-19	8.8E-18	Adenosylhomocysteinase
EC6.3.4.14	1.211	146.4	3.1E-19	8.8E-18	Biotin carboxylase
EC6.3.1.8	3.783	99.3	3.5E-19	9.9E-18	Glutathionylspermidine synthase
EC6.2.1.3	-0.812	193.0	3.6E-19	9.9E-18	Long-chain-fatty-acid--CoA ligase
EC2.3.2.-	2.110	213.5	3.9E-19	1.1E-17	NA
EC2.1.1.107	1.413	132.4	4.0E-19	1.1E-17	Uroporphyrinogen-III C-methyltransferase

EC3.6.3.3	1.308	136.7	4.9E-19	1.3E-17	Cadmium-exporting ATPase
EC5.3.3.8	2.858	145.6	5.0E-19	1.3E-17	Dodecenoyl-CoA isomerase
EC2.7.1.90	-1.565	208.4	1.3E-18	3.4E-17	Diphosphate--fructose-6-phosphate 1-phosphotransferase
EC6.2.1.30	-0.975	230.1	2.1E-18	5.3E-17	Phenylacetate--CoA ligase
EC4.1.1.32	-2.447	139.8	2.3E-18	5.8E-17	Phosphoenolpyruvate carboxykinase (GTP)
EC2.1.1.79	2.957	102.4	4.6E-18	1.1E-16	Cyclopropane-fatty-acyl-phospholipid synthase
EC1.7.1.4	2.064	126.2	6.0E-18	1.4E-16	Nitrite reductase (NAD(P)H)
EC2.1.1.37	-0.757	270.1	6.3E-18	1.5E-16	DNA (cytosine-5-)methyltransferase
EC1.1.1.35	1.549	228.2	8.0E-18	1.9E-16	3-hydroxyacyl-CoA dehydrogenase
EC1.2.1.16	2.141	114.3	8.1E-18	1.9E-16	Succinate-semialdehyde dehydrogenase (NAD(P)(+))
EC6.2.1.1	-0.900	221.5	2.0E-17	4.4E-16	Acetate--CoA ligase
EC3.2.1.55	-0.918	235.1	2.0E-17	4.4E-16	Alpha-N-arabinofuranosidase
EC6.1.1.18	-0.774	207.7	2.7E-17	6.0E-16	Glutamine--tRNA ligase
EC1.16.1.1	2.913	74.8	3.7E-17	8.0E-16	Mercury(II) reductase
EC2.3.1.16	2.108	179.8	5.1E-17	1.1E-15	Acetyl-CoA C-acyltransferase
EC2.7.1.144	2.108	104.0	5.1E-17	1.1E-15	Tagatose-6-phosphate kinase
EC6.3.2.2	2.060	94.2	6.8E-17	1.4E-15	Glutamate--cysteine ligase
EC1.4.1.4	-0.747	184.7	7.4E-17	1.5E-15	Glutamate dehydrogenase (NADP(+))
EC1.5.5.-	2.453	98.4	7.6E-17	1.5E-15	NA
EC3.1.1.45	3.656	83.9	8.4E-17	1.7E-15	Carboxymethylenebutenolidase
EC3.6.5.3	-0.343	307.7	1.2E-16	2.3E-15	Protein-synthesizing GTPase
EC3.6.1.11	1.901	113.7	1.7E-16	3.4E-15	Exopolyphosphatase
EC2.7.1.12	2.730	94.6	2.3E-16	4.5E-15	Gluconokinase
EC1.8.99.-	1.409	179.8	7.2E-16	1.4E-14	NA
EC3.2.1.122	3.600	49.4	7.4E-16	1.4E-14	Maltose-6'-phosphate glucosidase
EC1.7.1.7	3.015	89.2	9.4E-16	1.8E-14	GMP reductase
EC4.1.2.40	1.806	113.4	1.3E-15	2.5E-14	Tagatose-bisphosphate aldolase
EC4.1.2.9	2.391	189.4	1.4E-15	2.6E-14	Phosphoketolase
EC1.18.1.-	20.206	52.4	1.9E-15	3.4E-14	NA
EC1.5.99.8	1.654	141.0	2.5E-15	4.4E-14	Proline dehydrogenase
EC2.6.1.1	-0.737	212.8	3.9E-15	6.9E-14	Aspartate transaminase
EC5.1.3.9	1.734	80.9	3.9E-15	6.9E-14	N-acylglucosamine-6-phosphate 2-epimerase
EC3.5.99.4	20.170	54.4	5.8E-15	1.0E-13	N-isopropylammonium isopropylaminohydrolase
EC3.1.1.31	2.443	89.7	5.9E-15	1.0E-13	6-phosphogluconolactonase
EC1.2.1.10	0.667	201.3	7.2E-15	1.2E-13	Acetaldehyde dehydrogenase (acetylating)
EC2.2.1.2	1.484	107.8	7.3E-15	1.2E-13	Transaldolase
EC2.5.1.18	2.963	67.9	7.6E-15	1.3E-13	Glutathione transferase
EC1.11.1.6	1.309	241.0	9.7E-15	1.6E-13	Catalase
EC3.2.2.1	1.444	134.9	1.5E-14	2.5E-13	Purine nucleosidase
EC2.1.2.1	-0.752	174.1	1.8E-14	2.8E-13	Glycine hydroxymethyltransferase
EC2.7.7.58	20.132	52.1	1.8E-14	2.9E-13	(2,3-dihydroxybenzoyl)adenylate synthase
EC2.9.1.-	20.132	54.2	1.8E-14	2.9E-13	NA
EC3.6.3.32	3.511	56.5	1.9E-14	3.0E-13	Quaternary-amine-transporting ATPase
EC2.4.1.129	0.803	159.5	2.3E-14	3.6E-13	Peptidoglycan glycosyltransferase
EC2.7.7.59	2.506	86.1	2.4E-14	3.7E-13	[Protein-PII] uridylyltransferase
EC2.6.1.85	2.124	87.5	3.1E-14	4.7E-13	Aminodeoxychorismate synthase
EC6.3.2.3	4.105	65.3	3.4E-14	5.1E-13	Glutathione synthase
EC1.8.98.1	-1.171	205.7	5.4E-14	8.0E-13	CoB--CoM heterodisulfide reductase
EC3.2.1.4	-1.072	160.5	5.4E-14	8.0E-13	Cellulase
EC1.2.1.71	20.093	54.8	5.8E-14	8.4E-13	Succinylglutamate-semialdehyde dehydrogenase
EC2.7.11.5	20.093	62.5	5.8E-14	8.4E-13	[Isocitrate dehydrogenase (NADP(+))] kinase
EC3.2.1.28	2.907	94.2	6.1E-14	8.7E-13	Alpha,alpha-trehalase
EC3.4.14.11	2.302	131.0	6.6E-14	9.4E-13	Xaa-Pro dipeptidyl-peptidase
EC2.7.7.6	-0.239	329.2	8.1E-14	1.1E-12	DNA-directed RNA polymerase
EC2.7.1.95	1.917	185.0	8.5E-14	1.2E-12	Kanamycin kinase
EC3.4.14.4	-1.513	151.1	9.9E-14	1.4E-12	Dipeptidyl-peptidase III
EC4.1.1.47	4.070	60.9	1.0E-13	1.4E-12	Tartronate-semialdehyde synthase
EC5.99.1.3	-0.283	338.2	1.4E-13	1.9E-12	DNA topoisomerase (ATP-hydrolyzing)
EC3.6.1.40	3.446	78.6	1.7E-13	2.3E-12	Guanosine-5'-triphosphate,3'-diphosphate diphosphatase
EC5.4.2.8	-0.555	219.1	1.8E-13	2.4E-12	Phosphomannomutase
EC3.4.11.23	20.052	57.8	1.8E-13	2.4E-12	PepB aminopeptidase
EC3.1.3.26	20.052	53.6	1.8E-13	2.4E-12	4-phytase
EC5.4.4.2	1.818	113.1	2.8E-13	3.7E-12	Isochorismate synthase

EC1.6.1.1	4.034	71.0	3.2E-13	4.1E-12	NAD(P)(+) transhydrogenase (B-specific)
EC4.1.99.3	2.684	91.5	3.7E-13	4.8E-12	Deoxyribodipyrimidine photo-lyase
EC3.4.11.5	1.878	135.3	4.8E-13	6.2E-12	Prolyl aminopeptidase
EC3.1.4.16	1.270	121.5	1.6E-12	2.0E-11	2',3'-cyclic-nucleotide 2'-phosphodiesterase
EC2.7.1.73	19.965	49.0	1.8E-12	2.3E-11	Inosine kinase
EC1.1.-.-	1.081	196.9	2.4E-12	3.0E-11	NA
EC3.11.1.1	3.957	44.7	2.9E-12	3.6E-11	Phosphonoacetaldehyde hydrolase
EC3.2.1.51	-0.663	348.3	5.5E-12	6.8E-11	Alpha-L-fucosidase
EC1.14.11.17	20.918	47.5	5.8E-12	6.9E-11	Taurine dioxygenase
EC1.1.1.154	20.918	47.7	5.8E-12	6.9E-11	Ureidoglycolate dehydrogenase
EC5.5.-.-	19.918	49.0	5.8E-12	6.9E-11	NA
EC6.3.5.3	-0.384	248.5	7.1E-12	8.5E-11	Phosphoribosylformylglycinamide synthase
EC3.1.6.-	1.132	137.2	7.4E-12	8.7E-11	NA
EC1.3.1.76	1.740	100.0	8.2E-12	9.6E-11	Precorrin-2 dehydrogenase
EC4.2.1.30	1.740	92.6	8.2E-12	9.6E-11	Glycerol dehydratase
EC4.99.1.4	1.740	100.0	8.2E-12	9.6E-11	Sirohydrochlorin ferrochelatase
EC3.5.3.23	3.916	66.5	8.9E-12	1.0E-10	N-succinylarginine dihydrolase
EC1.9.3.1	1.674	140.7	9.9E-12	1.1E-10	Cytochrome-c oxidase
EC3.5.2.6	1.567	111.9	1.2E-11	1.4E-10	Beta-lactamase
EC2.7.1.113	2.972	113.2	1.3E-11	1.4E-10	Deoxyguanosine kinase
EC3.2.1.85	2.972	66.8	1.3E-11	1.4E-10	6-phospho-beta-galactosidase
EC6.2.1.17	2.972	65.1	1.3E-11	1.4E-10	Propionate--CoA ligase
EC2.5.1.49	-0.602	215.5	1.3E-11	1.5E-10	O-acetylhomoserine aminocarboxypropyltransferase
EC2.7.7.8	-0.477	206.1	1.4E-11	1.5E-10	Polyribonucleotide nucleotidyltransferase
EC2.7.7.9	1.090	155.3	1.7E-11	1.8E-10	UTP--glucose-1-phosphate uridylyltransferase
EC3.5.2.5	21.870	28.6	1.8E-11	2.0E-10	Allantoinase
EC4.2.1.79	3.874	55.2	2.7E-11	2.9E-10	2-methylcitrate dehydratase
EC6.3.1.2	-0.411	265.9	2.9E-11	3.1E-10	Glutamate--ammonia ligase
EC2.3.1.54	0.411	232.2	3.0E-11	3.1E-10	Formate C-acetyltransferase
EC3.4.15.5	-0.897	184.0	5.1E-11	5.4E-10	Peptidyl-dipeptidase Dcp
EC1.2.1.19	20.818	45.0	5.8E-11	6.0E-10	Aminobutyraldehyde dehydrogenase
EC4.1.1.85	20.818	40.9	5.8E-11	6.0E-10	3-dehydro-L-gulonate-6-phosphate decarboxylase
EC4.2.1.12	20.818	40.8	5.8E-11	6.0E-10	Phosphogluconate dehydratase
EC1.1.99.16	2.289	82.7	5.8E-11	6.0E-10	Malate dehydrogenase (acceptor)
EC4.4.1.8	1.530	142.0	5.9E-11	6.0E-10	Cystathione beta-lyase
EC3.5.4.4	1.487	93.0	6.0E-11	6.1E-10	Adenosine deaminase
EC2.7.2.2	1.044	141.1	7.4E-11	7.4E-10	Carbamate kinase
EC1.2.4.4	-1.756	127.5	9.7E-11	9.7E-10	3-methyl-2-oxobutanoate dehydrogenase (2-methylpropanoyl-transferring)
EC1.1.99.1	2.898	70.3	1.0E-10	1.0E-09	Choline dehydrogenase
EC1.14.12.17	2.898	62.2	1.0E-10	1.0E-09	Nitric oxide dioxygenase
EC1.3.1.74	-0.637	185.4	1.1E-10	1.1E-09	2-alkenal reductase
EC6.1.1.5	-0.380	225.1	1.1E-10	1.1E-09	Isoleucine-tRNA ligase
EC3.2.1.78	-20.430	83.0	1.2E-10	1.2E-09	Mannan endo-1,4-beta-mannosidase
EC5.1.3.14	-0.712	188.6	1.3E-10	1.2E-09	UDP-N-acetylglucosamine 2-epimerase
EC2.9.1.1	1.469	95.2	1.3E-10	1.2E-09	L-seryl-tRNA(Sec) selenium transferase
EC1.1.1.22	-0.757	175.8	1.3E-10	1.2E-09	UDP-glucose 6-dehydrogenase
EC3.2.1.8	-2.130	111.2	1.4E-10	1.4E-09	Endo-1,4-beta-xylanase
EC4.1.2.-	1.113	149.6	1.5E-10	1.4E-09	NA
EC5.4.99.2	-0.702	212.2	1.5E-10	1.4E-09	Methylmalonyl-CoA mutase
EC1.14.14.13	2.258	83.6	1.5E-10	1.4E-09	Alkanal monooxygenase (FMN-linked)
EC5.4.3.3	-1.901	129.2	1.6E-10	1.4E-09	Beta-lysine 5,6-aminomutase
EC2.4.1.5	19.764	63.9	1.8E-10	1.7E-09	Dextransucrase
EC3.3.2.1	1.951	86.4	2.0E-10	1.8E-09	Isochorismatase
EC3.6.3.27	0.810	171.0	2.1E-10	1.9E-09	Phosphate-transporting ATPase
EC3.1.1.1	-1.202	167.5	2.2E-10	2.0E-09	Carboxylesterase
EC3.1.3.6	2.648	61.3	2.2E-10	2.0E-09	3'-nucleotidase
EC1.14.12.19	3.783	52.1	2.5E-10	2.2E-09	3-phenylpropanoate dioxygenase
EC5.4.2.9	-19.392	101.6	2.7E-10	2.4E-09	Phosphoenolpyruvate mutase
EC1.6.5.3	-0.289	349.9	2.8E-10	2.5E-09	NADH dehydrogenase (ubiquinone)
EC3.2.1.14	1.249	177.0	3.1E-10	2.7E-09	Chitinase
EC2.3.1.157	0.812	157.2	3.1E-10	2.7E-09	Glucosamine-1-phosphate N-acetyltransferase
EC3.6.3.19	3.180	62.5	3.2E-10	2.8E-09	Maltose-transporting ATPase
EC3.5.1.78	3.180	64.9	3.2E-10	2.8E-09	Glutathionylspermidine amidase

EC2.7.2.7	-1.395	140.0	3.2E-10	2.8E-09	Butyrate kinase
EC1.20.4.1	0.959	132.1	3.9E-10	3.3E-09	Arsenate reductase (glutaredoxin)
EC2.6.1.66	2.226	85.6	3.9E-10	3.3E-09	Valine--pyruvate transaminase
EC6.3.3.3	2.226	72.4	3.9E-10	3.3E-09	Dethiobiotin synthase
EC2.7.1.45	-0.712	166.8	4.0E-10	3.3E-09	2-dehydro-3-deoxygluconokinase
EC1.6.4.-	1.703	105.9	4.2E-10	3.6E-09	NA
EC4.1.1.70	-1.970	110.0	4.3E-10	3.6E-09	Glutaconyl-CoA decarboxylase
EC6.1.1.4	-0.400	189.0	4.4E-10	3.7E-09	Leucine--tRNA ligase
EC3.2.2.23	1.632	113.0	5.0E-10	4.1E-09	DNA-formamidopyrimidine glycosylase
EC6.1.1.17	-0.553	222.3	5.1E-10	4.2E-09	Glutamate--tRNA ligase
EC1.17.7.1	-0.617	174.2	6.2E-10	5.1E-09	NA
EC2.6.1.62	1.265	130.7	7.5E-10	6.1E-09	Adenosylmethionine--8-amino-7-oxononanoate transaminase
EC1.21.4.2	0.880	220.6	8.3E-10	6.7E-09	Glycine reductase
EC1.14.--	1.719	108.0	8.8E-10	7.1E-09	NA
EC2.4.1.25	-0.501	264.4	9.1E-10	7.3E-09	4-alpha-glucanotransferase
EC2.7.1.56	1.130	108.4	1.0E-09	8.1E-09	1-phosphofructokinase
EC6.1.1.13	1.080	153.0	1.0E-09	8.1E-09	D-alanine--poly(phosphoribitol) ligase
EC3.1.2.1	-1.623	122.8	1.0E-09	8.1E-09	Acetyl-CoA hydrolase
EC2.1.1.13	-0.469	204.3	1.0E-09	8.2E-09	Methionine synthase
EC2.7.1.60	2.424	65.8	1.1E-09	8.7E-09	N-acylmannosamine kinase
EC6.2.1.5	1.412	126.7	1.2E-09	9.3E-09	Succinate--CoA ligase (ADP-forming)
EC1.1.1.27	0.918	202.8	1.2E-09	9.3E-09	L-lactate dehydrogenase
EC4.1.1.18	0.918	192.2	1.2E-09	9.3E-09	Lysine decarboxylase
EC1.2.1.22	1.522	105.8	1.2E-09	9.4E-09	Lactaldehyde dehydrogenase
EC1.2.1.70	1.474	83.1	1.2E-09	9.5E-09	Glutamyl-tRNA reductase
EC1.1.1.28	0.770	167.4	1.5E-09	1.1E-08	D-lactate dehydrogenase
EC2.6.1.57	2.571	72.9	1.7E-09	1.3E-08	Aromatic-amino-acid transaminase
EC3.5.1.47	2.279	67.1	1.8E-09	1.3E-08	N-acetyldiaminopimelate deacetylase
EC2.7.1.19	20.646	35.8	1.9E-09	1.4E-08	Phosphoribulokinase
EC2.3.3.10	19.646	60.7	1.9E-09	1.4E-08	Hydroxymethylglutaryl-CoA synthase
EC2.3.1.35	-0.764	206.6	1.9E-09	1.4E-08	Glutamate N-acetyltransferase
EC6.1.1.24	1.694	144.5	2.0E-09	1.5E-08	Glutamate--tRNA(Gln) ligase
EC4.2.1.40	1.694	119.8	2.0E-09	1.5E-08	Glucarate dehydratase
EC1.2.4.1	0.874	217.6	2.1E-09	1.5E-08	Pyruvate dehydrogenase (acetyl-transferring)
EC5.1.3.-	-0.782	172.3	2.2E-09	1.6E-08	NA
EC4.1.--	3.683	52.4	2.3E-09	1.6E-08	NA
EC3.5.1.28	0.403	216.6	2.4E-09	1.7E-08	N-acetyl muramoyl-L-alanine amidase
EC3.1.2.-	2.159	76.1	2.6E-09	1.9E-08	NA
EC1.6.5.5	1.527	100.4	2.6E-09	1.9E-08	NADPH:quinone reductase
EC2.7.1.58	3.089	63.6	2.7E-09	1.9E-08	2-dehydro-3-deoxygalactonokinase
EC1.1.5.3	0.575	204.4	2.9E-09	2.1E-08	NA
EC1.1.1.81	-1.584	127.8	3.5E-09	2.5E-08	Hydroxypyruvate reductase
EC2.6.1.82	2.060	83.0	3.5E-09	2.5E-08	Putrescine aminotransferase
EC1.1.1.267	-0.669	173.6	4.0E-09	2.8E-08	1-deoxy-D-xylulose-5-phosphate reductoisomerase
EC3.5.1.2	1.114	138.8	4.3E-09	3.0E-08	Glutaminase
EC4.1.3.1	2.530	72.1	4.6E-09	3.2E-08	Isocitrate lyase
EC2.4.1.7	1.454	122.8	5.6E-09	3.9E-08	Sucrose phosphorylase
EC1.2.1.39	20.582	31.4	5.9E-09	4.1E-08	Phenylacetaldehyde dehydrogenase
EC5.1.3.2	-0.462	192.4	6.1E-09	4.2E-08	UDP-glucose 4-epimerase
EC4.2.1.-	-0.482	207.9	6.5E-09	4.4E-08	NA
EC2.4.1.52	2.730	93.6	6.5E-09	4.4E-08	Poly(glycerol-phosphate) alpha-glucosyltransferase
EC3.5.1.11	2.124	77.9	6.6E-09	4.4E-08	Penicillin amidase
EC2.1.2.-	2.124	91.0	6.6E-09	4.4E-08	NA
EC3.4.21.83	1.837	85.7	6.7E-09	4.5E-08	Oligopeptidase B
EC2.4.1.15	3.628	37.8	6.8E-09	4.6E-08	Alpha,alpha-trehalose-phosphate synthase (UDP-forming)
EC2.1.1.48	3.041	51.8	7.9E-09	5.2E-08	rRNA (adenine-N(6)-)methyltransferase
EC3.4.21.102	-0.570	171.2	8.4E-09	5.5E-08	C-terminal processing peptidase
EC2.7.1.53	2.026	80.3	8.8E-09	5.8E-08	L-xylulokinase
EC3.1.21.5	-0.984	180.8	1.2E-08	7.7E-08	Type III site-specific deoxyribonuclease
EC2.7.4.7	0.764	160.8	1.2E-08	7.8E-08	Phosphomethylpyrimidine kinase
EC4.1.1.15	1.128	165.0	1.2E-08	7.8E-08	Glutamate decarboxylase
EC1.1.1.44	0.879	187.4	1.3E-08	8.4E-08	Phosphogluconate dehydrogenase (decarboxylating)
EC2.3.1.61	1.869	90.8	1.4E-08	8.8E-08	Dihydrolipoyllysine-residue succinyltransferase

EC2.8.1.1	1.869	95.7	1.4E-08	8.8E-08	Thiosulfate sulfurtransferase
EC2.3.3.13	-0.517	189.9	1.7E-08	1.1E-07	2-isopropylmalate synthase
EC2.7.1.59	20.513	31.0	1.9E-08	1.2E-07	N-acetylglucosamine kinase
EC3.1.27.6	20.513	41.9	1.9E-08	1.2E-07	Enterobacter ribonuclease
EC1.2.1.72	20.513	35.7	1.9E-08	1.2E-07	Erythrose-4-phosphate dehydrogenase
EC4.1.1.68	3.571	54.5	2.1E-08	1.3E-07	5-oxopent-3-ene-1,2,5-tricarboxylate decarboxylase
EC2.7.4.1	0.652	185.8	2.2E-08	1.4E-07	Polyphosphate kinase
EC1.1.1.6	1.367	91.9	2.3E-08	1.5E-07	Glycerol dehydrogenase
EC1.12.7.2	-0.664	197.7	2.7E-08	1.7E-07	Ferredoxin hydrogenase
EC5.3.1.25	-0.713	214.3	3.9E-08	2.4E-07	L-fucose isomerase
EC1.1.2.3	2.050	76.6	4.2E-08	2.6E-07	L-lactate dehydrogenase (cytochrome)
EC2.2.1.9	0.972	146.0	4.3E-08	2.7E-07	2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylic-acid
EC5.3.1.26	1.670	81.6	4.6E-08	2.8E-07	Galactose-6-phosphate isomerase
EC3.2.1.20	-0.481	193.9	4.8E-08	2.9E-07	Alpha-glucosidase
EC2.7.7.4	0.975	147.8	4.9E-08	3.0E-07	Sulfate adenylyltransferase
EC3.4.14.5	-0.770	184.2	5.8E-08	3.5E-07	Dipeptidyl-peptidase IV
EC1.5.1.7	-1.098	144.9	6.0E-08	3.6E-07	Saccharopine dehydrogenase (NAD(+), L-lysine-forming)
EC2.4.1.18	-0.438	205.3	6.1E-08	3.7E-07	1,4-alpha-glucan branching enzyme
EC2.7.4.2	2.935	69.3	6.5E-08	3.9E-07	Phosphomevalonate kinase
EC6.5.1.4	2.935	51.8	6.5E-08	3.9E-07	RNA-3'-phosphate cyclase
EC2.4.1.157	1.873	109.8	6.6E-08	3.9E-07	1,2-diacylglycerol 3-glucosyltransferase
EC2.7.8.6	-0.748	174.6	7.2E-08	4.3E-07	Undecaprenyl-phosphate galactose phosphotransferase
EC1.1.1.31	1.741	91.9	8.7E-08	5.2E-07	3-hydroxyisobutyrate dehydrogenase
EC1.4.3.21	2.396	72.6	9.1E-08	5.4E-07	Primary-amine oxidase
EC4.1.1.33	2.396	77.7	9.1E-08	5.4E-07	Diphosphomevalonate decarboxylase
EC1.3.99.2	-1.047	130.3	9.7E-08	5.7E-07	Butyryl-CoA dehydrogenase
EC1.2.2.2	2.011	77.4	1.0E-07	6.0E-07	Pyruvate dehydrogenase (cytochrome)
EC4.99.1.1	2.011	67.3	1.0E-07	6.0E-07	Ferrochelatase
EC1.4.99.1	2.011	65.2	1.0E-07	6.0E-07	D-amino-acid dehydrogenase
EC3.5.1.19	2.011	72.9	1.0E-07	6.0E-07	Nicotinamidase
EC2.7.1.48	-0.574	173.2	1.1E-07	6.3E-07	Uridine kinase
EC1.2.1.8	1.596	86.5	1.1E-07	6.3E-07	Betaine-aldehyde dehydrogenase
EC3.4.13.9	0.793	150.0	1.3E-07	7.2E-07	Xaa-Pro dipeptidase
EC3.2.1.89	-1.191	137.4	1.3E-07	7.4E-07	Arabinogalactan endo-1,4-beta-galactosidase
EC4.2.1.7	-0.832	139.9	1.3E-07	7.7E-07	Altronate dehydratase
EC1.18.1.3	2.584	63.6	1.4E-07	8.0E-07	Ferredoxin-NAD(+) reductase
EC3.2.1.23	-0.254	290.5	1.5E-07	8.5E-07	Beta-galactosidase
EC3.4.17.-	-2.206	88.5	1.6E-07	8.9E-07	NA
EC2.5.1.48	1.068	138.6	1.6E-07	8.9E-07	Cystathionine gamma-synthase
EC1.2.7.7	-19.015	80.3	1.7E-07	9.5E-07	3-methyl-2-oxobutanoate dehydrogenase (ferredoxin)
EC1.14.1.-	3.446	47.0	1.9E-07	1.0E-06	NA
EC2.7.7.47	2.878	54.0	1.9E-07	1.0E-06	Streptomycin 3"-adenylyltransferase
EC1.1.1.79	20.359	28.9	1.9E-07	1.1E-06	Glyoxylate reductase (NADP(+))
EC1.3.3.3	20.359	30.2	1.9E-07	1.1E-06	Coproporphyrinogen oxidase
EC3.1.1.32	20.359	30.6	1.9E-07	1.1E-06	Phospholipase A(1)
EC1.3.1.28	20.359	31.1	1.9E-07	1.1E-06	2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase
EC1.14.11.-	2.082	72.4	2.0E-07	1.1E-06	NA
EC3.1.13.1	2.082	84.9	2.0E-07	1.1E-06	Exoribonuclease II
EC2.6.1.19	1.187	114.3	2.5E-07	1.4E-06	4-aminobutyrate transaminase
EC1.1.1.95	-0.478	183.7	2.5E-07	1.4E-06	Phosphoglycerate dehydrogenase
EC2.6.1.52	-0.566	188.0	2.7E-07	1.4E-06	Phosphoserine transaminase
EC6.2.1.-	-0.539	240.3	2.7E-07	1.5E-06	NA
EC1.2.1.-	1.199	133.4	2.7E-07	1.5E-06	NA
EC3.2.1.22	-0.467	188.0	2.8E-07	1.5E-06	Alpha-galactosidase
EC2.7.1.40	0.469	216.7	3.5E-07	1.9E-06	Pyruvate kinase
EC2.8.4.1	-18.957	79.7	3.8E-07	2.0E-06	Coenzyme-B sulfoethylthiotransferase
EC5.1.3.8	-1.320	116.3	4.0E-07	2.1E-06	N-acetylglucosamine 2-epimerase
EC5.4.2.7	0.583	167.0	4.6E-07	2.4E-06	Phosphopentomutase
EC2.4.2.14	-0.397	220.0	4.7E-07	2.5E-06	Amidophosphoribosyltransferase
EC2.1.1.144	2.818	38.9	5.4E-07	2.8E-06	Trans-aconitate 2-methyltransferase
EC2.7.4.23	3.377	44.8	5.6E-07	2.9E-06	Ribose 1,5-bisphosphate phosphokinase
EC3.1.7.2	1.190	128.2	5.8E-07	3.0E-06	Guanosine-3',5'-bis(diphosphate) 3'-diphosphatase
EC3.5.4.12	-0.779	148.6	5.8E-07	3.0E-06	dCMP deaminase

EC2.7.1.87	20.272	41.8	6.3E-07	3.2E-06	Streptomycin 3"-kinase
EC3.6.3.33	20.272	30.7	6.3E-07	3.2E-06	Vitamin B12-transporting ATPase
EC4.1.3.30	1.928	70.1	6.4E-07	3.3E-06	Methylisocitrate lyase
EC1.2.1.60	2.296	53.4	6.5E-07	3.3E-06	5-carboxymethyl-2-hydroxymuconic-semialdehyde dehydrogenase
EC4.1.1.41	-0.808	155.2	6.7E-07	3.4E-06	Methylmalonyl-CoA decarboxylase
EC6.1.1.12	-0.383	209.7	7.0E-07	3.5E-06	Aspartate--tRNA ligase
EC3.6.1.-	0.118	271.9	8.1E-07	4.1E-06	NA
EC3.1.3.11	0.476	181.7	8.8E-07	4.5E-06	Fructose-bisphosphatase
EC2.7.7.13	-1.500	98.9	9.5E-07	4.8E-06	Mannose-1-phosphate guanyllyltransferase
EC3.6.1.45	1.401	115.2	1.1E-06	5.7E-06	UDP-sugar diphosphatase
EC2.3.1.29	-0.674	151.5	1.1E-06	5.7E-06	Glycine C-acetyltransferase
EC2.4.1.83	-1.090	123.5	1.2E-06	5.9E-06	Dolichyl-phosphate beta-D-mannosyltransferase
EC2.1.1.51	1.194	87.9	1.3E-06	6.6E-06	rRNA (guanine-N(1)-)methyltransferase
EC1.4.1.13	-0.248	331.0	1.3E-06	6.6E-06	Glutamate synthase (NADPH)
EC1.4.1.14	-0.248	331.0	1.3E-06	6.6E-06	Glutamate synthase (NADH)
EC3.2.1.91	-2.375	86.2	1.5E-06	7.4E-06	Cellulose 1,4-beta-cellulobiosidase
EC3.1.21.7	2.753	43.8	1.5E-06	7.5E-06	Deoxyribonuclease V
EC2.7.2.15	3.303	40.7	1.7E-06	8.2E-06	Propionate kinase
EC3.5.3.9	2.242	59.3	1.7E-06	8.3E-06	Allantoate deiminase
EC3.6.1.41	2.242	57.8	1.7E-06	8.3E-06	Bis(5'-nucleosyl)-tetraphosphatase (symmetrical)
EC2.7.1.15	0.750	150.3	1.9E-06	9.1E-06	Ribokinase
EC1.1.1.169	0.895	138.6	1.9E-06	9.3E-06	2-dehydropantoate 2-reductase
EC4.2.1.80	20.176	29.3	2.0E-06	9.7E-06	2-oxopent-4-enoate hydratase
EC6.3.1.11	20.176	28.4	2.0E-06	9.7E-06	Glutamate--putrescine ligase
EC3.5.1.96	20.176	29.5	2.0E-06	9.7E-06	Succinylglutamate desuccinylase
EC1.17.4.2	-0.280	316.5	2.0E-06	9.7E-06	Ribonucleoside-triphosphate reductase
EC3.1.26.4	-0.459	167.0	2.1E-06	9.9E-06	Ribonuclease H
EC6.1.1.19	-0.389	173.1	2.5E-06	1.2E-05	Arginine--tRNA ligase
EC4.2.1.46	-0.408	172.2	2.7E-06	1.3E-05	dTDP-glucose 4,6-dehydratase
EC3.1.21.1	2.412	52.1	2.9E-06	1.4E-05	Deoxyribonuclease I
EC1.5.1.12	0.738	174.7	3.0E-06	1.4E-05	1-pyrroline-5-carboxylate dehydrogenase
EC2.7.1.-	0.417	193.1	3.1E-06	1.5E-05	NA
EC4.4.1.-	1.942	78.6	3.1E-06	1.5E-05	NA
EC1.1.1.17	1.213	109.4	3.3E-06	1.5E-05	Mannitol-1-phosphate 5-dehydrogenase
EC3.5.1.-	-0.386	167.0	3.5E-06	1.6E-05	NA
EC1.12.98.1	-2.845	80.7	4.1E-06	1.9E-05	Coenzyme F420 hydrogenase
EC4.2.1.24	0.884	119.5	4.3E-06	2.0E-05	Porphobilinogen synthase
EC4.2.99.20	2.684	42.9	4.3E-06	2.0E-05	2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate synthase
EC2.7.7.23	0.558	157.7	4.7E-06	2.2E-05	UDP-N-acetylglucosamine diphosphorylase
EC3.5.3.6	0.917	114.7	4.8E-06	2.2E-05	Arginine deiminase
EC2.6.1.83	-0.744	181.7	5.0E-06	2.3E-05	LL-diaminopimelate aminotransferase
EC4.2.3.5	-0.474	178.7	5.1E-06	2.3E-05	Chorismate synthase
EC3.4.21.107	1.559	90.3	5.3E-06	2.4E-05	Peptidase Do
EC1.8.4.11	1.431	82.7	5.4E-06	2.5E-05	Peptide-methionine (S)-S-oxide reductase
EC3.1.3.27	1.511	78.1	5.4E-06	2.5E-05	Phosphatidylglycerophosphatase
EC6.1.1.22	-0.444	178.1	6.0E-06	2.7E-05	Asparagine--tRNA ligase
EC3.2.1.35	0.961	145.6	6.0E-06	2.7E-05	Hyaluronoglucosaminidase
EC1.1.1.90	2.019	70.8	6.1E-06	2.7E-05	Aryl-alcohol dehydrogenase
EC3.2.2.8	2.019	63.0	6.1E-06	2.7E-05	Ribosylpyrimidine nucleosidase
EC6.1.1.21	-0.426	157.4	6.4E-06	2.9E-05	Histidine--tRNA ligase
EC1.5.3.-	20.071	28.8	6.6E-06	2.9E-05	NA
EC3.5.1.25	0.462	159.6	7.5E-06	3.3E-05	N-acetylglucosamine-6-phosphate deacetylase
EC1.14.14.5	1.891	63.4	7.6E-06	3.4E-05	Alkanesulfonate monooxygenase
EC6.3.5.1	-0.456	211.5	7.7E-06	3.4E-05	NAD(+) synthase (glutamine-hydrolyzing)
EC6.1.1.1	-0.436	162.0	8.4E-06	3.7E-05	Tyrosine--tRNA ligase
EC2.3.1.48	1.259	116.1	8.9E-06	3.9E-05	Histone acetyltransferase
EC2.5.1.6	-0.336	207.5	9.0E-06	4.0E-05	Methionine adenosyltransferase
EC6.4.1.1	0.424	299.5	9.2E-06	4.0E-05	Pyruvate carboxylase
EC3.8.1.-	-0.933	118.7	9.2E-06	4.0E-05	NA
EC2.1.1.86	-18.679	64.5	9.9E-06	4.3E-05	Tetrahydromethanopterin S-methyltransferase
EC3.2.1.93	0.847	120.5	1.1E-05	4.6E-05	Alpha,alpha-phosphotrehalase
EC1.1.1.88	1.632	96.8	1.1E-05	4.7E-05	Hydroxymethylglutaryl-CoA reductase
EC2.2.1.1	-0.275	202.6	1.2E-05	5.0E-05	Transketolase

EC2.7.9.3	0.825	145.5	1.2E-05	5.0E-05	Selenide, water dikinase
EC1.3.1.-	0.652	137.4	1.2E-05	5.0E-05	NA
EC3.1.13.5	1.431	89.4	1.2E-05	5.0E-05	Ribonuclease D
EC4.1.1.8	1.431	87.0	1.2E-05	5.0E-05	Oxalyl-CoA decarboxylase
EC6.2.1.26	1.060	119.3	1.3E-05	5.3E-05	o-succinylbenzoate--CoA ligase
EC6.4.1.3	-0.513	183.1	1.3E-05	5.5E-05	Propionyl-CoA carboxylase
EC1.2.99.7	-1.313	126.7	1.3E-05	5.6E-05	Aldehyde dehydrogenase (FAD-independent)
EC3.5.1.53	-1.372	107.7	1.3E-05	5.7E-05	N-carbamoylputrescine amidase
EC2.7.10.-	0.912	125.9	1.4E-05	5.9E-05	NA
EC2.5.1.8	-0.479	158.4	1.4E-05	6.0E-05	tRNA isopentenyltransferase
EC1.1.1.205	-0.335	218.4	1.5E-05	6.1E-05	IMP dehydrogenase
EC3.1.1.5	1.190	91.1	1.5E-05	6.2E-05	Lysophospholipase
EC4.1.3.40	3.136	43.8	1.5E-05	6.2E-05	Chorismate lyase
EC6.3.-.-	3.136	38.6	1.5E-05	6.2E-05	NA
EC4.2.1.1	0.828	143.0	1.5E-05	6.3E-05	Carbonate dehydratase
EC3.4.19.5	1.208	124.0	1.6E-05	6.7E-05	Beta-aspartyl-peptidase
EC1.2.7.5	0.677	180.0	1.8E-05	7.2E-05	Aldehyde ferredoxin oxidoreductase
EC6.1.1.10	-0.302	202.2	1.8E-05	7.3E-05	Methionine--tRNA ligase
EC6.1.1.9	-0.268	208.2	1.8E-05	7.3E-05	Valine--tRNA ligase
EC6.1.1.7	-0.285	203.2	1.8E-05	7.4E-05	Alanine--tRNA ligase
EC2.4.1.1	-0.265	254.3	1.8E-05	7.4E-05	Phosphorylase
EC4.1.3.-.-	-0.633	136.7	1.8E-05	7.5E-05	NA
EC4.6.1.12	-0.696	155.4	1.9E-05	7.6E-05	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase
EC2.7.2.3	-0.384	190.3	2.0E-05	8.0E-05	Phosphoglycerate kinase
EC3.2.1.10	0.648	198.1	2.0E-05	8.2E-05	Oligo-1,6-glucosidase
EC2.6.1.81	2.279	48.0	2.1E-05	8.4E-05	Succinylornithine transaminase
EC1.13.11.15	19.953	26.1	2.1E-05	8.6E-05	3,4-dihydroxyphenylacetate 2,3-dioxygenase
EC1.13.11.16	19.953	24.6	2.1E-05	8.6E-05	3-carboxyethylcatechol 2,3-dioxygenase
EC2.3.1.1	-0.490	175.1	2.2E-05	8.8E-05	Amino-acid N-acetyltransferase
EC5.3.1.12	-0.549	159.5	2.2E-05	8.9E-05	Glucuronate isomerase
EC4.1.2.21	1.654	82.4	2.3E-05	9.2E-05	2-dehydro-3-deoxy-6-phosphogalactonate aldolase
EC3.2.1.96	1.654	66.3	2.3E-05	9.2E-05	Mannosyl-glycoprotein endo-beta-N-acetylglucosaminidase
EC4.3.1.17	0.384	212.5	2.4E-05	9.3E-05	L-serine ammonia-lyase
EC1.97.1.9	0.738	144.4	2.4E-05	9.4E-05	Selenate reductase
EC5.3.1.22	1.585	80.0	2.5E-05	9.7E-05	Hydroxypyruvate isomerase
EC1.1.1.34	1.527	112.8	2.5E-05	1.0E-04	Hydroxymethylglutaryl-CoA reductase (NADPH)
EC5.3.1.1	-0.468	173.8	2.6E-05	1.0E-04	Triose-phosphate isomerase
EC2.7.7.10	0.685	180.4	3.0E-05	1.2E-04	UTP--hexose-1-phosphate uridylyltransferase
EC2.5.1.-.-	0.435	205.2	3.1E-05	1.2E-04	NA
EC5.3.1.23	-1.066	109.5	3.1E-05	1.2E-04	S-methyl-5-thioribose-1-phosphate isomerase
EC3.6.1.1	-0.344	208.9	3.2E-05	1.3E-04	Inorganic diphosphatase
EC2.1.1.113	-18.551	72.6	3.4E-05	1.3E-04	Site-specific DNA-methyltransferase (cytosine-N(4)-specific)
EC1.2.7.1	-0.715	160.7	3.5E-05	1.3E-04	Pyruvate synthase
EC4.2.1.47	-0.773	150.6	3.5E-05	1.3E-04	GDP-mannose 4,6-dehydratase
EC1.1.1.29	-0.733	146.6	3.5E-05	1.3E-04	Glycerate dehydrogenase
EC4.2.2.2	-1.704	94.6	3.5E-05	1.4E-04	Pectate lyase
EC2.7.1.89	3.041	35.9	4.5E-05	1.7E-04	Thiamine kinase
EC2.8.1.2	3.041	38.5	4.5E-05	1.7E-04	3-mercaptopyruvate sulfurtransferase
EC3.5.1.94	3.041	41.7	4.5E-05	1.7E-04	Gamma-glutamyl-gamma-aminobutyrate hydrolase
EC1.1.1.23	-0.485	171.7	5.2E-05	2.0E-04	Histidinol dehydrogenase
EC2.1.1.35	1.351	66.9	5.2E-05	2.0E-04	tRNA (uracil-5')-methyltransferase
EC4.1.1.49	-0.402	180.3	5.2E-05	2.0E-04	Phosphoenolpyruvate carboxykinase (ATP)
EC1.2.99.5	-1.852	92.9	5.5E-05	2.1E-04	Formylmethanofuran dehydrogenase
EC2.7.7.1	1.480	86.7	5.6E-05	2.1E-04	Nicotinamide-nucleotide adenylyltransferase
EC3.6.3.20	1.480	63.8	5.6E-05	2.1E-04	Glycerol-3-phosphate-transporting ATPase
EC2.7.7.-.-	0.293	226.0	5.8E-05	2.2E-04	NA
EC4.1.1.82	-2.629	77.7	5.9E-05	2.2E-04	Phosphonopyruvate decarboxylase
EC3.2.1.31	-0.829	128.7	5.9E-05	2.2E-04	Beta-glucuronidase
EC2.7.7.24	-0.462	165.5	6.0E-05	2.2E-04	Glucose-1-phosphate thymidyltransferase
EC2.6.1.42	-0.447	158.6	7.0E-05	2.6E-04	Branched-chain-amino-acid transaminase
EC1.1.3.21	19.820	23.4	7.0E-05	2.6E-04	Glycerol-3-phosphate oxidase
EC3.1.3.23	19.820	25.6	7.0E-05	2.6E-04	Sugar-phosphatase
EC3.2.1.41	-0.621	203.8	7.0E-05	2.6E-04	Pullulanase

EC3.1.21.4	-0.597	192.0	7.4E-05	2.7E-04	Type II site-specific deoxyribonuclease
EC2.1.1.148	-1.277	108.1	7.5E-05	2.7E-04	Thymidylate synthase (FAD)
EC2.5.1.1	-1.127	107.8	7.6E-05	2.8E-04	Dimethylallyltransferase
EC2.7.1.36	1.991	73.5	7.6E-05	2.8E-04	Mevalonate kinase
EC3.5.4.25	-0.606	179.6	7.9E-05	2.9E-04	GTP cyclohydrolase II
EC2.5.1.61	0.888	94.1	8.1E-05	2.9E-04	Hydroxymethylbilane synthase
EC3.1.26.-	-0.610	147.8	8.1E-05	2.9E-04	NA
EC2.5.1.7	-0.294	213.0	8.6E-05	3.1E-04	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
EC1.6.1.2	0.722	162.4	8.8E-05	3.2E-04	NAD(P)(+)-transhydrogenase (AB-specific)
EC3.1.2.14	1.837	66.9	9.3E-05	3.3E-04	Oleoyl-[acyl-carrier-protein] hydrolase
EC3.2.1.25	-0.683	150.8	1.1E-04	3.9E-04	Beta-mannosidase
EC3.2.1.24	0.476	162.0	1.1E-04	4.1E-04	Alpha-mannosidase
EC1.1.1.58	-0.718	136.9	1.1E-04	4.1E-04	Tagaturose reductase
EC1.1.1.284	1.344	79.2	1.1E-04	4.1E-04	S-(hydroxymethyl)glutathione dehydrogenase
EC2.7.7.38	-0.882	140.3	1.2E-04	4.2E-04	3-deoxy-manno-octulosonate cytidyltransferase
EC3.1.1.-	0.550	162.5	1.2E-04	4.2E-04	NA
EC2.7.1.107	1.549	55.5	1.2E-04	4.2E-04	Diacylglycerol kinase
EC2.7.8.7	1.549	57.0	1.2E-04	4.2E-04	Holo-[acyl-carrier-protein] synthase
EC4.1.1.12	1.431	70.4	1.2E-04	4.3E-04	Aspartate 4-decarboxylase
EC3.1.26.8	1.485	88.2	1.2E-04	4.3E-04	Ribonuclease M5
EC2.3.1.8	0.374	178.7	1.3E-04	4.7E-04	Phosphate acetyltransferase
EC3.4.21.92	-0.376	206.4	1.4E-04	4.7E-04	Endopeptidase Clp
EC2.7.2.1	-0.308	190.8	1.4E-04	4.8E-04	Acetate kinase
EC3.4.21.26	-1.481	108.8	1.4E-04	4.9E-04	Prolyl oligopeptidase
EC1.11.1.15	0.580	150.4	1.4E-04	4.9E-04	Peroxiredoxin
EC5.3.1.14	-0.603	159.2	1.4E-04	5.0E-04	L-rhamnose isomerase
EC1.4.3.-	2.124	44.4	1.5E-04	5.1E-04	NA
EC3.1.1.53	-0.655	171.6	1.5E-04	5.2E-04	Sialate O-acetyltransferase
EC6.1.1.11	-0.349	164.7	1.5E-04	5.2E-04	Serine-tRNA ligase
EC3.4.24.64	1.163	97.5	1.6E-04	5.5E-04	Mitochondrial processing peptidase
EC3.2.1.81	-18.350	59.0	1.8E-04	6.1E-04	Beta-agarase
EC2.4.2.29	-0.363	176.2	1.8E-04	6.2E-04	tRNA-guanine transglycosylase
EC4.3.1.15	0.872	101.3	1.8E-04	6.2E-04	Diaminopropionate ammonia-lyase
EC3.4.21.116	-0.861	151.2	1.8E-04	6.3E-04	SpoIVB peptidase
EC3.1.1.3	-1.459	95.5	1.9E-04	6.4E-04	Triacylglycerol lipase
EC1.18.6.1	-1.362	103.4	1.9E-04	6.5E-04	Nitrogenase
EC2.7.1.49	0.562	158.6	2.0E-04	6.8E-04	Hydroxymethylpyrimidine kinase
EC4.1.1.19	0.459	203.8	2.1E-04	7.1E-04	Arginine decarboxylase
EC4.3.1.19	0.461	154.5	2.2E-04	7.5E-04	Threonine ammonia-lyase
EC3.1.25.-	20.666	20.2	2.3E-04	7.8E-04	NA
EC3.6.1.26	20.666	21.6	2.3E-04	7.8E-04	CDP-diacylglycerol diphosphatase
EC2.1.4.1	19.666	25.4	2.3E-04	7.8E-04	Glycine amidinotransferase
EC3.1.11.3	19.666	22.7	2.3E-04	7.8E-04	Exodeoxyribonuclease (lambda-induced)
EC3.1.4.14	19.666	22.7	2.3E-04	7.8E-04	[Acyl-carrier-protein] phosphodiesterase
EC3.4.23.49	19.666	22.7	2.3E-04	7.8E-04	OmpT
EC5.3.4.1	1.654	55.7	2.5E-04	8.3E-04	Protein disulfide-isomerase
EC1.4.4.2	-0.288	259.2	2.5E-04	8.4E-04	Glycine dehydrogenase (decarboxylating)
EC2.7.1.5	-0.520	143.5	2.6E-04	8.6E-04	Rhamnulokinase
EC3.1.4.-	2.348	36.3	2.6E-04	8.7E-04	NA
EC3.6.1.7	1.431	60.6	2.7E-04	8.8E-04	Acylphosphatase
EC6.---	1.492	62.3	2.7E-04	8.8E-04	NA
EC2.3.1.40	0.843	134.5	2.7E-04	8.8E-04	Acyl-[acyl-carrier-protein]-phospholipid O-acyltransferase
EC3.6.3.17	0.211	262.0	2.7E-04	8.8E-04	Monosaccharide-transporting ATPase
EC2.7.1.16	-0.420	175.7	2.7E-04	8.9E-04	Ribulokinase
EC6.3.5.5	-0.163	277.9	2.8E-04	9.0E-04	Carbamoyl-phosphate synthase (glutamine-hydrolyzing)
EC1.3.1.34	0.686	127.2	2.8E-04	9.2E-04	2,4-dienoyl-CoA reductase (NADPH)
EC1.1.1.69	-0.689	139.6	3.0E-04	9.7E-04	Gluconate 5-dehydrogenase

Table S5.

E.C.s encoded by genes whose representation is significantly different between herbivorous and carnivorous microbiomes. * = Poisson model coefficient. †= Akaike Information Criterion, smaller values denote better agreement between model and data. ‡= Benjamini-Hochberg corrected. §="NA" E.C. with no specific annotation.

Amino Acid	Biosynthetic Reactions	Degradative Reactions	Reversible Reactions
Alanine	No difference	No difference	
Arginine	2.3.1.1, 2.3.1.35 (Both increased in Herbivores)	3.5.3.6 (Carnivores)	
Asparagine	No difference	No difference	
Aspartic Acid	No difference	4.1.1.12 (Carnivores)	2.6.1.1 (Herbivores)
Cysteine	2.5.1.49 (Herbivores)	No difference	
Glutamic Acid	1.4.1.4, 1.4.1.13, 1.4.1.14 (All increased in Herbivores)	4.1.1.15, 2.6.1.19, 1.2.1.16 (All increased in Carnivores)	
Glutamine	6.3.1.2 (Herbivore)	3.5.1.2 (Carnivores)	
Glycine	2.1.2.1 (Herbivores)	No difference	
Histidine	1.1.1.23 (Herbivores)	No difference	
Isoleucine	No difference	1.2.7.7, 1.2.4.4, 1.3.99.2 (All increased in Herbivores)	2.6.1.42 (Herbivores)
Leucine	No difference	1.2.7.7, 1.2.4.4 (Both increased in Herbivores)	2.6.1.42 (Herbivores)
Lysine	1.5.1.7, 3.5.1.- (Both increased in Herbivores)	4.1.1.8 (Carnivores)	
Methionine	2.1.1.13 (Herbivores), S-adenosyl-L-methionine cycle [2.5.1.6,2.1.1.37,3.3.1.1](All increased in Herbivores)	No difference	2.1.1.14 (Carnivores)
Phenylalanine	4.2.3.5 (Herbivores)	No difference	2.6.1.1 (Herbivores), 2.6.1.57 (Carnivores)
Proline	No difference	1.5.99.8, 1.5.1.12 (Both increased in Carnivores)	
Serine	1.1.1.95, 2.6.1.52 (Both increased in Herbivores)	4.3.1.17, 4.3.1.19 (Both increased in Carnivores)	
Threonine	No difference	4.3.1.19 (Carnivores)	
Tryptophan	4.2.3.5 (Herbivores), 4.2.1.20(Herbivores)	1.14.13.- (Carnivores), 2.5.1.- (Carnivores)	
Tyrosine	4.2.3.5 (Herbivores)	No difference	2.6.1.1 (Herbivores), 2.6.1.57 (Carnivores)
Valine	No difference	1.2.7.7, 1.2.4.4, 1.3.99.2 (All increased in Herbivores)	2.6.1.42 (Herbivores), 2.6.1.66 (Carnivores)
Herbivore Total:	12	3	6
Carnivore Total:	0	9	4

Table S6.

Summary of differences in amino acid metabolism between herbivore and carnivore microbiomes.

SampleID	BMI (kg/m ²)	Total Carbo- hydrate (g/day)	Total Protein (g/day)	Insoluble Dietary Fiber (g/day)
CRBM.02	18.5	227.3	68.4	24.6
CRBM.03	20.1	302.9	94	48.8
CRBM.04	18.5	217.6	92.8	42.7
CRBM.05	19.4	378.2	69.3	62.7
CRBM.06	19.7	276.5	71.5	50.8
CRBM.07	19.2	130.1	56.4	18.5
CRBM.08	16.7	216.7	62.1	40
CRBM.09	18.7	257.2	116.6	37
CRBM.10	19.4	102	145.6	30.7
CRBM.11	18.3	253.1	64.5	19.7
CRBM.12	20.3	195.7	78.8	38.6
CRBM.13	17.5	169.6	61.9	29.8
CRBM.14	22.5	168.4	41.6	22.9
CRBM.15	22.2	125.3	70.7	15.3
CRBM.16	20.1	341.9	88	77.7
CRBM.19	19.5	194.7	69.6	32.1
CRBM.20	18.6	216	47.3	20.5
CRBM.21	20.6	203.7	79.1	17.2

Table S7.

Metadata on 18 calorie restricted humans included in this study, including host BMI and intake of major food categories.

SampleID	Total 16S rRNA V2 reads (De-noised, Chimera Checked)	Total OTUs (97% ID)	Shannon's Index*
CRBM.02	2159	350	6.52
CRBM.03	3279	421	6.71
CRBM.04	2365	407	6.70
CRBM.05	3614	379	6.38
CRBM.06	2716	443	6.77
CRBM.07	18818	917	6.81
CRBM.08	3240	365	6.14
CRBM.09	2431	328	6.34
CRBM.10	3610	337	6.32
CRBM.11	2311	360	6.32
CRBM.12	2695	418	6.96
CRBM.13	2350	303	6.05
CRBM.14	2855	316	5.77
CRBM.15	2317	383	6.37
CRBM.16	3274	215	5.18
CRBM.19	2274	327	6.05
CRBM.20	3415	440	6.77
CRBM.21	1826	302	6.09

* average of 50 iterations at 1500 reads per sample

Table S8.

16S rRNA sequencing statistics from calorie restricted humans

SampleID	Sequencing chemistry	Total shotgun reads (quality filtered, dereplicated)	Mean read length (\pm standard deviation) [nt]	Number of reads with KEGG Orthology group (KO) assignment	Total E.C. assignments	Number of reads with MEROPS assignment	Number of reads with CAZy assignment*
CRBM.02	FLX	46535	226 (\pm 45)	15017	9811	1042	1234
CRBM.03	FLX	92140	222 (\pm 46)	30057	20019	2161	2381
CRBM.04	FLX	35735	227 (\pm 42)	10993	7294	771	896
CRBM.05	FLX	152547	217 (\pm 44)	49629	33199	3466	4426
CRBM.06	FLX	33800	232 (\pm 36)	10973	7116	717	775
CRBM.07	FLX	37360	229 (\pm 37)	12032	7789	831	1010
CRBM.08	FLX	50597	229 (\pm 39)	16129	10818	1256	1421
CRBM.09	FLX	40147	219 (\pm 51)	12690	8484	991	1139
CRBM.10	FLX	47895	233 (\pm 36)	15895	10502	1217	1338
CRBM.11	FLX	46573	226 (\pm 39)	16159	10507	1101	1410
CRBM.12	FLX	40883	228 (\pm 37)	12931	8533	914	836
CRBM.13	FLX	55303	229 (\pm 36)	19975	13048	1244	1500
CRBM.14	FLX	65833	221 (\pm 43)	22484	15038	1576	1809
CRBM.15	FLX	44298	222 (\pm 51)	13150	8795	875	980
CRBM.16	FLX	36113	230 (\pm 36)	11676	7656	797	1160
CRBM.19	FLX	50444	220 (\pm 50)	15282	10116	1078	1281
CRBM.20	FLX	57998	233 (\pm 34))	19511	12663	1260	1233
CRBM.21	FLX	43107	233 (\pm 36)	13794	8995	996	1027

*All reads assigned as Glycoside Hydrolase, Polysaccharide Lyase, or Carbohydrate Esterase

Table S9.

Shotgun pyrosequencing datasets obtained from fecal DNA prepared from calorie restricted humans: statistics.

Table S10A

SampleID	Total number of reads with hit in nr (%)	Total number of reads with no hit (%)
CRBM.02	42480 (91.3%)	4055 (8.7%)
CRBM.03	84019 (91.2%)	8121 (8.8%)
CRBM.04	32607 (91.3%)	3128 (8.8%)
CRBM.05	138600 (90.9%)	13947 (9.1%)
CRBM.06	30859 (91.3%)	2941 (8.7%)
CRBM.07	34201 (91.5%)	3159 (8.5%)
CRBM.08	47210 (93.3%)	3387 (6.7%)
CRBM.09	36603 (91.2%)	3544 (8.8%)
CRBM.10	44443 (92.8%)	3452 (7.2%)
CRBM.11	43336 (93.1%)	3237 (7.0%)
CRBM.12	37205 (91.0%)	3678 (9.0%)
CRBM.13	51679 (93.5%)	3624 (6.6%)
CRBM.14	60795 (92.4%)	5038 (7.7%)
CRBM.15	40014 (90.3%)	4284 (9.7%)
CRBM.16	33524 (92.8%)	2589 (7.2%)
CRBM.19	45123 (89.5%)	5321 (10.5%)
CRBM.20	53238 (91.8%)	4760 (8.2%)
CRBM.21	39416 (91.4%)	3691 (8.6%)

Table S10B

SampleID	Bacteria	Eukarya	Archaea	Viruses	Cellular Organism / Root	Not assigned
CRBM.02	91.93%	0.89%	ND	ND	4.37%	2.82%
CRBM.03	87.31%	1.92%	0.018%	ND	8.43%	2.32%
CRBM.04	89.26%	1.16%	ND	ND	5.98%	3.60%
CRBM.05	88.43%	2.07%	0.007%	ND	7.80%	1.70%
CRBM.06	87.71%	1.53%	0.078%	ND	6.51%	4.16%
CRBM.07	88.03%	1.35%	0.015%	0.015%	6.73%	3.86%
CRBM.08	91.87%	0.97%	ND	ND	4.66%	2.50%
CRBM.09	89.98%	0.82%	0.459%	ND	5.71%	3.03%
CRBM.10	90.23%	1.24%	ND	ND	5.51%	3.02%
CRBM.11	92.74%	0.70%	ND	ND	3.94%	2.62%
CRBM.12	86.52%	1.56%	ND	ND	7.98%	3.94%
CRBM.13	90.32%	1.16%	0.010%	0.010%	5.71%	2.79%
CRBM.14	92.06%	1.19%	ND	ND	4.43%	2.32%
CRBM.15	89.67%	1.00%	1.242%	ND	5.00%	3.09%
CRBM.16	91.83%	0.78%	ND	ND	4.30%	3.10%
CRBM.19	89.32%	1.45%	ND	ND	5.99%	3.24%
CRBM.20	88.49%	1.91%	0.009%	ND	6.64%	2.95%
CRBM.21	90.71%	1.17%	ND	ND	4.74%	3.38%

"ND" = No reads detected in this taxonomic branch

Table S10.

Shotgun pyrosequencing datasets obtained from fecal DNA prepared from calorie restricted humans: phylogenetic assignments. (A) Summary of total hits against the NCBI non-redundant (nr) database. (B) Percentage of all reads assigned to the major phylogenetic divisions (normalized to total number of reads with hit in NCBI nr database).

Principal Coordinates Category	Diet Category	Regression Results						
		Intercept	Slope	P-Value	Adjusted P- Value (Bonferroni)	R2	Adjusted R2	F-Statistic
16S rRNA OTU Counts (Bray-Curtis)	Total Carbo- hydrate (g/day)	0.239	-0.001	0.045	0.136	0.23	0.18	4.72
16S rRNA OTU Counts (Bray-Curtis)	Total Protein (g/day)	0.152	-0.002	0.233	0.699	0.09	0.03	1.54
16S rRNA OTU Counts (Bray-Curtis)	Insoluble Dietary Fiber (g/day)	0.218	-0.006	0.004	0.013	0.41	0.37	11.02
16S rRNA (Weighted UniFrac)	Total Carbo- hydrate (g/day)	-0.023	0.000	0.741	2.223	0.01	-0.06	0.11
16S rRNA (Weighted UniFrac)	Total Protein (g/day)	-0.059	0.001	0.410	1.230	0.04	-0.02	0.72
16S rRNA (Weighted UniFrac)	Insoluble Dietary Fiber (g/day)	-0.033	0.001	0.493	1.480	0.03	-0.03	0.49
16S rRNA (Unweighted UniFrac)	Total Carbo- hydrate (g/day)	0.064	0.000	0.575	1.724	0.02	-0.04	0.33
16S rRNA (Unweighted UniFrac)	Total Protein (g/day)	0.019	0.000	0.869	2.608	0.00	-0.06	0.03
16S rRNA (Unweighted UniFrac)	Insoluble Dietary Fiber (g/day)	0.084	-0.002	0.270	0.809	0.08	0.02	1.31
KO	Total Carbo- hydrate (g/day)	-0.012	0.000	0.778	2.333	0.01	-0.06	0.08
KO	Total Protein (g/day)	-0.103	0.001	0.010	0.030	0.35	0.31	8.52
KO	Insoluble Dietary Fiber (g/day)	-0.041	0.001	0.156	0.469	0.12	0.07	2.21

Table S11.

Results of regression analysis comparing position on Principal Coordinate 1 with host dietary intake.

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