Title: Achieving pan-microbiome biological insights via the dbBact knowledge base

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Abstract

16S rRNA amplicon sequencing provides a relatively cheap culture-independent method for studying the microbial world. Thousands of studies have examined microbial populations in various habitats, yet a global pan-microbiome perspective integrating these studies is still missing. Here we introduce dbBact, an open wiki-like bacterial knowledge base that combines information from hundreds of studies across diverse habitats, creating a collaborative central repository amenable to subsequent analysis. 16S rRNA amplicon sequence variants (ASVs) are manually extracted from each study and assigned multiple ontology-based terms. dbBact currently contains about 850 studies, covering more than 1,300,000 associations between 315,000 ASVs and 3,500 ontology terms. We demonstrate how dbBact can lead to better understanding of underlying bacterial communities and the formulation of novel biological hypotheses. dbBact can be accessed using a website (http://dbbact.org), plugins for qiime2 and Calour, and programmatically, using a REST-API.

Introduction

Bacteria play an important role in the Earth's ecosystem, having a total biomass higher than that of all vertebrates and fish, second only to plants (1). The introduction of 16S rRNA amplicon sequencing as a means for molecular identification has enabled a culture-independent view of such ecosystems (2). Combined with massively parallel sequencing technologies and DNA barcodes (3), 16S rRNA sequencing provides relatively cheap and accurate microbial profiling. This, in turn, has led to a huge surge in the number of 16S rRNA studies examining microbial populations in habitats ranging across oceans (4), soil (5), plants (6), animals (7), and large cross-sectional human studies (8–10).

A severe limitation when combining insights from multiple microbiome studies is the complexity of the underlying bacterial populations, ranging from tens of different bacteria in a single saliva sample (11), to thousands in a single soil sample (12). In addition, although the total number of different bacteria is large (e.g., ~300000 unique 16S rRNA sequences of length 90bp appear in the Earth Microbiome Project (12)), the number of taxonomic names for describing these bacteria is

much smaller (about 3500-4000 unique genera and 20000 unique species names appear in NCBITAX (13) and in the Encyclopedia of Life (14)). Moreover, grouping bacteria in higher taxonomic levels may not always maintain the basic habitat properties of many bacteria (12). Therefore, reaching cross-study biological insights should preferably be based on 16S rRNA amplicon sequences rather than on taxonomy.

Recently, amplicon sequence variants (ASVs) derived using denoising methods such as Deblur (15), DADA2 (16) and UNOISE2 (17) have been introduced as an alternative to OTU picking for identifying bacteria in a given sample. Such denoising methods provide an objective identification of each bacterial sequence in the sample (i.e., independent of external databases or additional bacteria/samples in the experiment), as well as high sequence resolution (a single nucleotide difference in the sequenced region is identified as a different ASV). Therefore, ASVs may serve as cross-study identifiers for bacteria, i.e., a bacterium in different studies will result in the same ASV, even when the studies are processed separately and denoised using different methods (15, 18).

In this paper, we introduce dbBact, a knowledge base for reaching cross-experiment biological insights. dbBact is based on manually collecting genotype-phenotype associations between ASVs and relevant conditions. For clarity, "reserved" dbBact words appear in italics: surveyed studies are referred to as *experiments*, stored ASVs are *sequences*, phenotypes are ontology-based *terms*, and genotype-phenotype associations are called *annotations*.

Currently, dbBact contains more than 850 studies in various habitats, covering more than 1,300,000 associations between 315,000 ASVs and 3,500 ontology terms. For retrieval, dbBact provides two query types: a single *sequence*/FASTA file query, asking what is known about these *sequences* (Figure 1), and a query contrasting two FASTA files, searching for dbBact *terms* significantly enriched in either of the groups, analogously to gene ontology enrichment analysis (19, 20) (Figure 2). By examining the ontology *terms* associated with each *sequence*, users can gain insights regarding the biology associated with ASVs of interest.

dbBact differs from other microbial databases in several aspects: (a) *Manual annotation*: dbBact phenotype-genotype associations are extracted using manual analysis, in contrast to microbial data repositories, such as SRA/EBI, Qiita (21), HMP DACC (10, 22), MGnify (23), FoodmicrobioNet (24), and redBIOM (25), which provide raw experimental data and metadata. In dbBact, the human expert understands the experimental setting and identifies abundant bacteria in different study

groups, detects contaminants, etc., and these associations are uploaded. (b) *Unlimited scope*: dbBact accepts studies across all habitats, unlike databases that are highly limited in scope, e.g., human-disease-focused databases (MicroPhenoDB (26), gutMDisorder (27), Disbiome database (28), Peryton (29), and BugSigDB (30)), or other context-specific databases (database of the healthy mouse microbiome (31) or the sponge microbiome project (32)). (c) *Volume and potential growth*: to date, the number of studies in dbBact is ~40% higher than in Qiita, and the number of ASVs is comparable to those in the Earth Microbiome Project. New studies are continually added by the dbBact team. Additionally, as a wiki-like database, we encourage the microbiome community to contribute to dbBact. (d) *Structured genotype/phenotype search*: observations are uploaded at the ASV level, allowing queries of specific *sequence*s and sub*sequence*s. In addition, as phenotypes are designated by *terms* derived from multiple ontologies, subsequent querying allows for "cross-sectioning" of the data. For example, *sequences* associated with Crohn's disease and ulcerative colitis both originate from the DOID ontology (33), and will be recalled when querying their "parent" *term,* "inflammatory bowel disease." (e) *Harmonizing studies performed using different variable regions*: as uploaded studies may be *sequence*d using different 16S rRNA variable regions, stored *sequence*s are "linked" through the SILVA database of full-length 16S rRNA genes (34), facilitating cross-region queries. For example, when submitting a query *sequence* from V1-V2, dbBact seeks the matching full-length 16S rRNA genes in SILVA, then extracts their V4 region, and subsequently retrieves relevant *annotation*s. (f) *Data analysis*: dbBact provides a set of statistical tools for analyzing new studies and for generating novel biological hypotheses.

Apart from accessing dbBact through its website (http://dbbact.org), it is possible to integrate dbBact in microbiome analysis pipelines through the Qiime2 (35) and Calour (36) plugins, and programmatic query submission is available through the dbBact REST-API interface. A weekly snapshot of the complete dbBact SQL database is available for download, and can be used for local analysis.

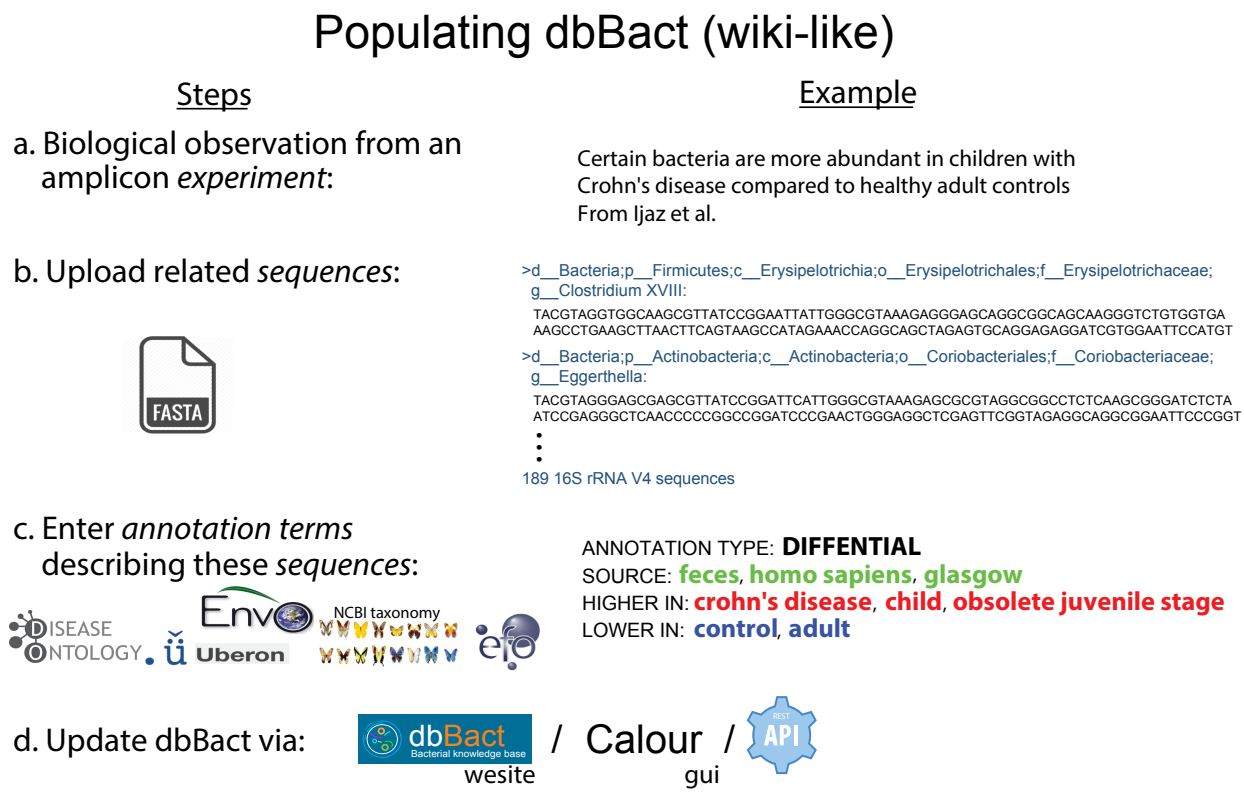


Figure 1: **Adding entries to dbBact**. Users add new entries in a wiki-like way, by uploading study results. **a.** For example, analyzing data from Ijaz et al. (37), we identified 189 ASVs that are more abundant in fecal samples of Scottish children with Crohn's disease compared to healthy controls (see Methods section). **b.** These ASVs are uploaded as a FASTA file. **c.** Associations between ASVs and phenotypes are called *annotations*, which are created by assigning a set of ontology *terms* and predicates that characterize the context. The 189 *sequences* were *annotated* as "DIFFERENTIAL," i.e., more abundant in children with Crohn's disease ("HIGHER IN" *terms*), compared to healthy controls ("LOWER IN" *terms*). The general background *terms* common to both groups, i.e., "homo sapiens," "feces" and "glasgow" are designated by "SOURCE." *Terms* may be selected from several ontologies (e.g., DOID (33), ENVO (38, 39), GAZ (40), UBERON (41), EFO (42), and NCBI Taxonomy (13)), allowing easy and precise *annotations*. **d.** Uploading *annotations* may be performed either through the dbBact website, dedicated clients (i.e., Calour (36)) or by REST-API. For clarity, the following nomenclature holds throughout the manuscript where "reserved" words appear in italics (e.g., *experiment*, *sequence*, *annotation, term*), predicates appear in all caps (e.g., "HIGHER IN," "LOWER IN," "SOURCE"), and specific *term* names follow the ontology convention of being lower case (e.g., "homo sapiens").

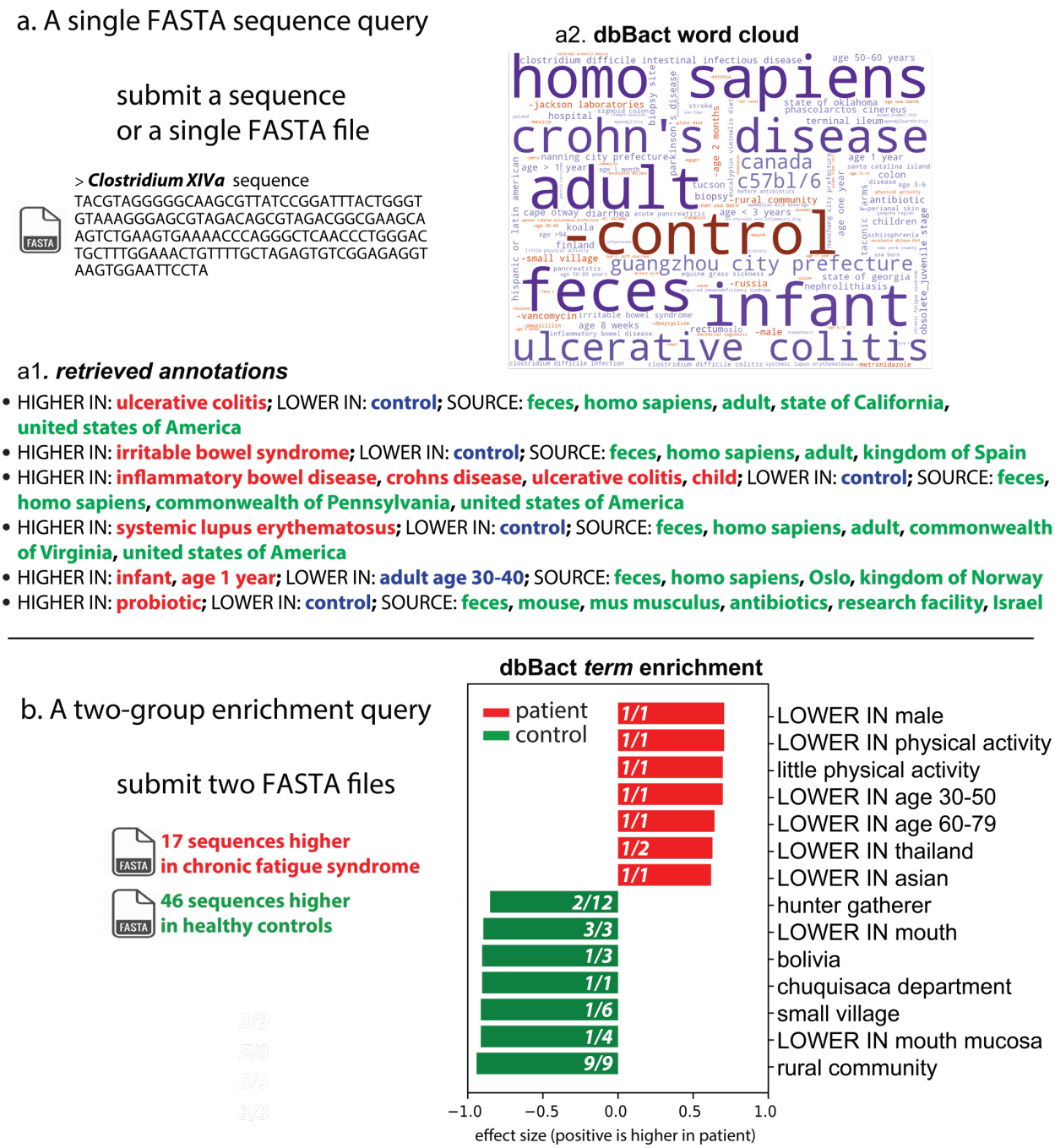


Figure 2: **Two basic query types: a.** Uploading a FASTA file of *sequences* results in a list of the most relevant *annotations* containing these *sequences*, and a "word cloud" of best matching *terms*. In this example, a V4 *sequence* of Clostridium XIVa, which is highly abundant in fecal samples of chronic fatigue syndrome patients (CSF) (Giloteaux et al., 2016), was submitted. Panel **a1** provides representative *annotations* containing the query sequence (the full list of ~150 *annotations* appears in Supplementary File 3). dbBact found

this *sequence* to be higher in the disease group than in healthy controls in several studies (ulcerative colitis, irritable bowel disease, and lupus), and in antibiotic-treated mice supplemented with probiotics (last *annotation* arising from (43)). Panel **a2** displays the word cloud summarizing the *terms* associated with the query *sequence*, where size corresponds to a *term*'s F-score, while color designates the associated predicate (blue for "SOURCE"/"HIGHER IN" *terms*, and red color preceded by a minus sign corresponds to "LOWER IN" *terms*). Hence, this Clostridium XIVa query *sequence* is associated with human feces in dysbiosis states of "crohn's disease," "ulcerative colitis," "diarrhea," and "c. difficile infection" (a full list of F-scores per term appears in Supplementary File S7). **b.** When uploading two FASTA files, dbBact identifies enriched *terms* characterizing each group. For example, 17 and 46 *sequences* were submitted, corresponding to differentially abundant *sequences* higher in chronic fatigue syndrome (CFS) patients and in healthy controls, respectively. Bar lengths show the normalized rank-mean difference for the top significantly enriched *terms* in either bacterial group. *Term* enrichment is based on a non-parametric rank mean test with FDR<0.1 using dsFDR (see term enrichment analysis section in Methods). The numbers in the bar of each *term* correspond to the number of dbBact *experiments* in which the *term* is significantly different between the two *sequence* groups (numerator), and the total of dbBact *experiments* containing the *term* (denominator). *Sequences* that were more abundant in the CFS group are enriched in *terms* related to low physical activity, while healthy controls are enriched in *terms* related to rural and less industrialized communities.

Results

dbBact: Scope and comprehensiveness

For the sake of reproducibility, we used dbBact release 2021.05 for all analyses presented in the Results section. This release contains approximately 260,000 unique bacterial amplicon *sequences*, an amount that is on par with the 300,000 sequences observed by the Earth Microbiome Project (12). *Sequences* arise from over 780 unique *experiments*, i.e., studies from which observations were added (Figure 3a). Over 5500 dbBact *annotations* associate these *sequences* with various phenotypes using ontology derived *terms*. As each annotation typically includes many *sequences*, this results in over 960,000 unique genotype-phenotype associations.

General statistics of dbBact

The ~780 dbBact *experiments* cover a wide range of habitats (Figure S1a), geographic regions (Figure S1b), plant and animal hosts (Figure S1c), human body sites (Figure S1d), and human diseases (Figure 3b). For example, 149 *experiments* cover diseases, of which 86 are of an anatomical entity (e.g., Crohn's disease or ulcerative colitis), and seven more are defined as metabolic diseases. The most abundant dbBact *terms* are "united states of america," "homo sapiens," and "feces," each appearing in over 1000 *annotations* arising from more than 150 different *experiments.* Most of the other *terms* appear in less than twenty *experiments* (Figure 3c). The most prevalent bacterial *sequence* is *E. coli,* appearing in over 900 *annotations* from over 300 *experiments* (Figure 3d). Although this could reflect the universality of *E. coli* in various habitats, it may also be due to potential contaminations (44), a reason that may also explain the high prevalence of *Staphylococcus* (appearing in over 200 dbBact *experiments*). The number of *experiments* per *sequence* follows a power law distribution, with a majority of *sequences* appearing in a single *experiment*, yet over 80,000 *sequences* were observed in more than one *experiment* and 7000 *sequences* appeared in at least ten *experiments* (Figure 3d).

dbBact allows the upload of *sequences* from several commonly used regions (V1-V2, V3-V4 or V4; see Table S2 for a list of primers). Upon upload, *sequences* from different regions are "linked" through their full-length 16S rRNA sequence in the SILVA database (34) (see Inter-region querying section in Methods). When submitting query *sequences* from one region, dbBact retrieves all *annotations* containing the corresponding *sequences* across all regions (including, naturally, the region from which the query was provided). To demonstrate the usefulness of such "linking," Figure S2 provides several examples of V1-V2 and V3-V4 *sequence* queries that are successfully characterized based solely on "linked" V4 *sequences.*

Comprehensiveness of dbBact

Intra-dbBact estimates. To estimate the comprehensiveness of dbBact, we tested how many bacterial *sequences* typical of a specific environment (e.g., human feces) have *annotations* arising from more than one *experiment*. We selected *sequences* having an *annotation* of type "COMMON" (i.e., present in more than half of the samples in an *experiment*) for each of several *terms*, and measured the fraction of these *sequences* that have *annotations* from <u>another</u> dbBact *experiment* (Figure 3e). For example, there are 97 *experiments* having a "COMMON IN homo

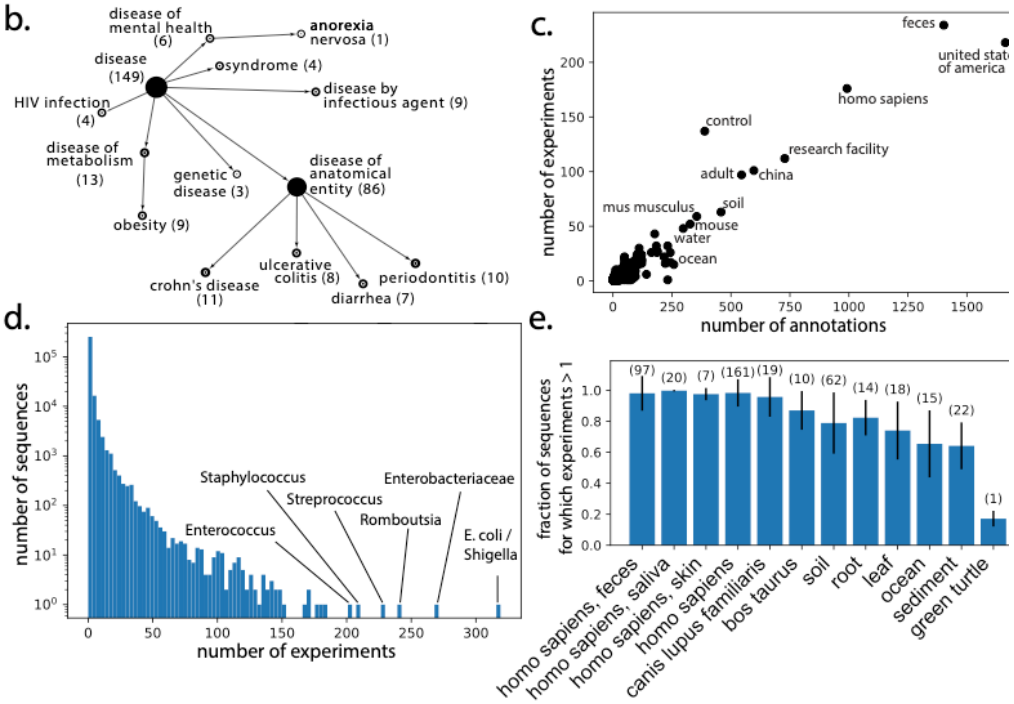
sapiens feces" *annotation*. Iterating over each of these *annotations*, about 98% of the associated *sequences* appear in more than one *experiment*. Hence, fecal bacteria are already well covered by dbBact. A similar level of "coverage" occurs for several other human-related *terms* and for dogs, where almost all *sequences* were observed in more than a single *experiment*. Regarding the *terms* "cow," "soil," "root," and "leaf," about 80% of the *sequences* appear in more than a single *experiment*, whereas the "coverage" of "green turtle" is much lower, indicating that additional experiments are required to capture its full bacterial diversity.

Out-of-sample comprehensiveness. As another example of comprehensiveness, dbBact was tested in a source tracking task, i.e., identifying the host or niche of a sample based on its bacterial composition. Hägglund et al. collected samples from either sewage influent or from freshwater, as well as feces sampled from several animals (rabbit, cat, wild boar, dog, cow and deer), aiming to find unique bacterial footprints of each source (45). We used all *sequences* present in more than 1/3 of the samples from each group as queries to dbBact resulting in word clouds describing each sample group (Figure 3f). In almost all cases, the notable *terms* in each word cloud were indicative of the sources of the samples, e.g., *sus scrofa* for the wild boar fecal sample, allowing accurate source tracking. The only exception was cat fecal samples, which were detected as a combination of cat, dog, and human, probably because of the small number of cat fecal samples present in the current dbBact release.

a.

dbBact
Bacterial knowledge base
release 2021.05

experiments	annotations	sequences	associations between sequences & annotations
784	5556	260242	960257



f.

Figure 3: **Knowledge base scope and comprehensiveness**. **a.** Scope of dbBact release 2021.05 (used for the analysis presented in this paper). **b.** The number of *experiments* for representative disease categories based on the DOID ontology. **c.** Scatter plot of the total number of *annotations* and *experiments* in which each dbBact *term* appears. **d.** Histogram of the number of *experiments* in which each dbBact *sequence* appears. **e.** Knowledge base comprehensiveness. The fraction of "COMMON" *sequences* from each *experiment* that have been annotated in additional *experiments* is shown for various *terms*. The number of experiments containing the *term* is designated above each bar. **f.** Comprehensiveness in a source tracking task. *Sequences* from eight sample types from Hägglund et al. were blindly submitted to dbBact. Their word clouds clearly display the sources of the samples (shown by the matched cartoon). *Term* sizes correspond to the F-score of each *term,* combined for all *sequences* present in > 0.3 of the samples (for each sample type).

The advantage of sequence-based associations

Results of 16S rRNA profiling experiments comprise a list of ASVs found in each sample and their abundances. Corroboration of these results with other microbiome studies is typically performed by searching published studies mentioning the taxonomy of these sequences. In many cases, however, such text-based mining may be limited because of constraints in taxonomic assignment. First, taxonomy is far from being full, e.g., species-level assignment is missing for about 80% of 16S rRNA sequences in Greengenes (46), and about 35% of the Greengenes sequences lack a genus assignment (47). Second, in many cases the same assigned taxonomy may be associated with vastly different phenotypes. As observed by the Earth Microbiome Project, bacteria of the same genus may be present in vastly different habitats, whereas specific sequences are associated with a certain habitat (12). This phenomenon underscores the importance of sequence-based association as provided by dbBact. For example, both *sequences* in Figure 4a belong to the genus *Blautia*, hence taxonomy-based associations may conclude that they play similar "roles" and are associated with the same phenotype. But querying dbBact with each of these two *sequences* results in a strikingly different picture, which we refer to as a "good" and "bad" *Blautia*. The "good" *Blautia* is more abundant in healthy controls than in patients of type 1 diabetes (T1D), Crohns' disease (CD), inflammatory bowel disease (IBD), diarrhea, and kidney

stones (Figure 4a), whereas the "bad" *Blautia* is more prevalent in patients suffering from IBD, CD and ulcerative colitis (Figure 4b).

Collecting all "disease" related dbBact annotations shows that the "bad" *Blautia* is "HIGHER IN" in the disease group (compared to controls) in 8/9 disease *annotations* associated with it, whereas the "good" *Blautia* is "LOWER IN" in the disease group (compared to controls) in 22/24 disease *annotations* (Figure 4c). Therefore, sequence-based analysis provides a solid genotype-to-phenotype association compared to taxonomy-based associations.

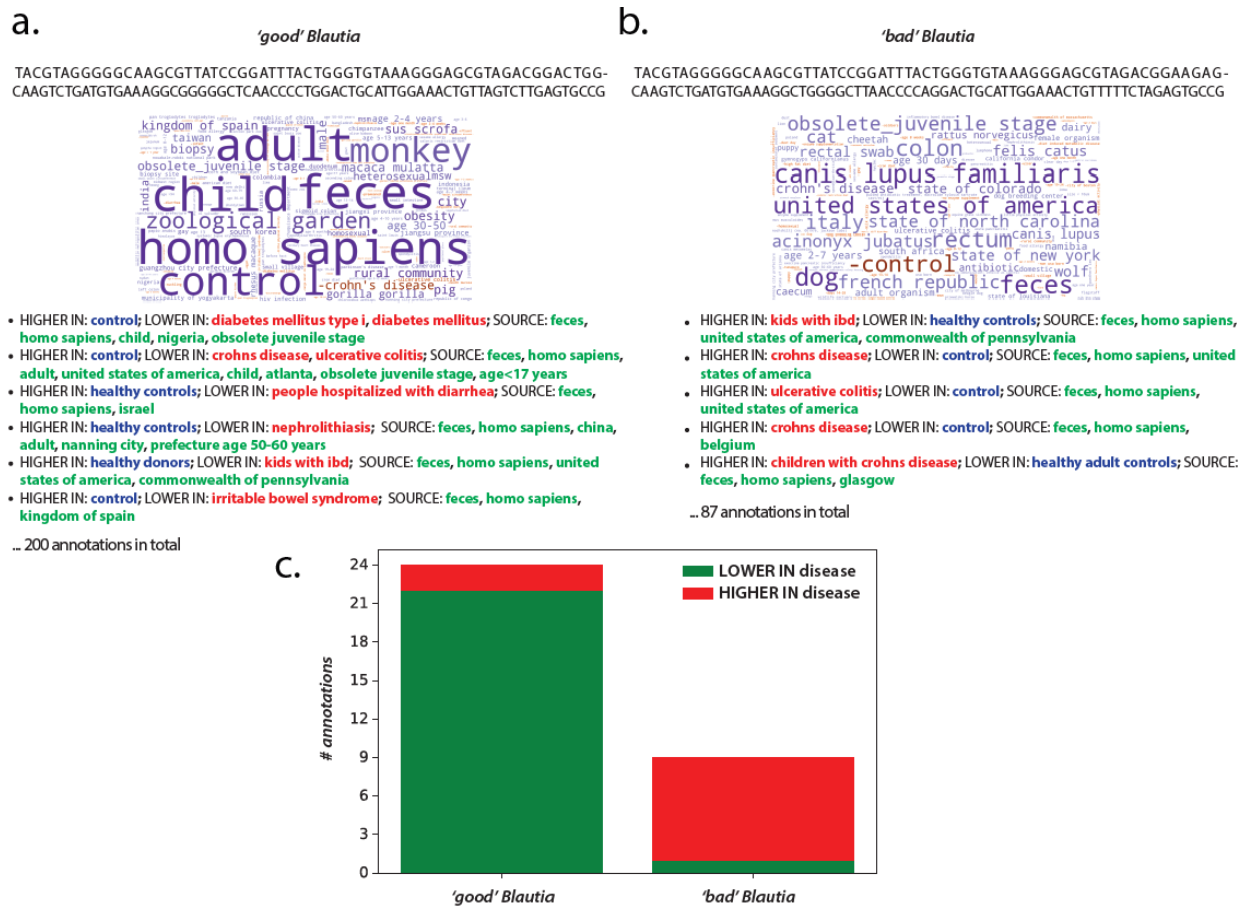


Figure 4: Taxonomy may be misleading. **a.** Two *sequences* of the genus *Blautia*, that differ by nine bases over the 150bp Illumina read of the 16S rRNA V4 region are associated with opposite phenotypes, as discovered by dbBact. The two word clouds and annotations for each *sequence*, display "opposite" associations with disease. The left *sequence* is more prevalent in healthy subjects ("good" *Blautia*), whereas the other is highly abundant in a series of disease-related *annotations*. Such differences can be traced through dbBact, but are completely missed by a taxonomy-based analysis. **b.** The number of disease-related

annotations for the two *Blautia sequences* across dbBact displays an opposite trend of being low and high in disease, for the "good" and "bad" *Blautia*, respectively. The total number of *annotations* in dbBact 2021.05 associated with the "good" and "bad" *Blautia sequences* is 377 and 124, respectively.

dbBact provides a pan-microbiome view: Detailed example

dbBact may add another layer to data analysis in microbiome studies by identifying commonalities between different conditions and diseases, generating novel biological hypotheses. To demonstrate such a pan-microbiome analysis, we use data from a study comparing subjects consuming an American diet to a calorie restricted diet (48), and demonstrate the use of dbBact *term* enrichment. Fecal samples from two groups of lean individuals (BMI<25) who followed either an American diet (AMER) or a caloric restriction diet (CR) were selected. Standard analysis with FDR set to 0.1 (see "standard analysis" section in Methods) identified 28 and 141 bacterial *sequences* significantly more abundant in AMER and CR cohorts, respectively (Figure 3a). For clarity, we refer to these groups of *sequences* as S-AMER and S-CR, respectively. Figure 3b shows the internal "transformation" performed by dbBact from a heatmap of bacterial abundances to a heatmap of association scores of *terms* for each *sequence* (columns in Figure 3a-b are aligned and correspond to the same *sequences*). For example, the *term* "high BMI," appears in almost all S-CR *sequences*, while it is almost absent in S-AMER *sequences*. These association scores in Figure 3b are then used as input to a non-parametric differential abundance test (see Methods section "statistical analysis in dbBact"), identifying *terms* significantly enriched in each of the two sequence groups (Figure 3c). Results indicate that *sequences* in the S-CR group are associated with *terms* related to low BMI ("low bmi," "LOWER IN high bmi") and with rural/undeveloped habitats ("LOWER IN united states of america," "small village") (see Supplementary File 4 for the full list of enriched terms). By contrast, bacteria from the S-AMER group have a significantly higher number of *annotations* related to high BMI ("LOWER IN low bmi," "high bmi") and urban/modernized habitats ("state of oklahoma," "LOWER IN rural community," "LOWER IN small village").

Thus, although participants from both diet groups were lean, certain aspects of the underlying microbiome were associated with high and low BMI bacteria, for AMER and CR, respectively. Additionally, bacteria enriched in CR vs. AMER tend to be associated with rural/undeveloped

habitats, which may indicate an adaptation of some bacteria found in rural communities to a low-calorie/higher vegetable diet content.

To further confirm the relationship between diet and BMI, we collected all *sequences* across dbBact having a "high bmi" annotation, resulting in 319 *sequences*. The overlap between these *sequences* and the S-AMER and S-CR groups is shown in Figure 3d (left). Although 93% (26/28) of S-AMER *sequences* overlap with "high bmi"-associated *sequences*, the overlap of S-CR *sequences* is 11% (15/141), i.e., a much larger fraction of S-AMER *sequences* is associated with high BMI. An analogous Venn diagram for the *term* "low bmi" displays an overlap of 54% (15/28) and 78% (110/141) of S-AMER and S-CRON bacteria with "low bmi"-associated bacteria in dbBact, respectively (Figure 3d left). As participants from both CR and AMER groups were lean (BMI<25), one may hypothesize that the effect of BMI on the microbial composition, observed in various studies, is due to dietary differences rather than the high BMI phenotype.

Remark regarding spurious/irrelevant terms: dbBact release 2021.05 contains *annotations* of over 2500 unique *terms*, some appearing only in a few *experiments*. As a result, word clouds and bar plots may often include seemingly odd *terms*. For example, the *term* "state of oklahoma" in Figure 5d is significantly enriched in S-AMER, a fact that seems implausible. This *term* appears only in two dbBact *experiments*, one of which compared a rural community in Peru to an urban community in Oklahoma (49). Hence, *annotations* from this *experiment* mentioned the *term* "state of oklahoma" together with more relevant *terms* (e.g., "rural community") which, in turn, caused its inclusion. As dbBact continues to grow, such "transient" irrelevant inductions are expected to diminish.

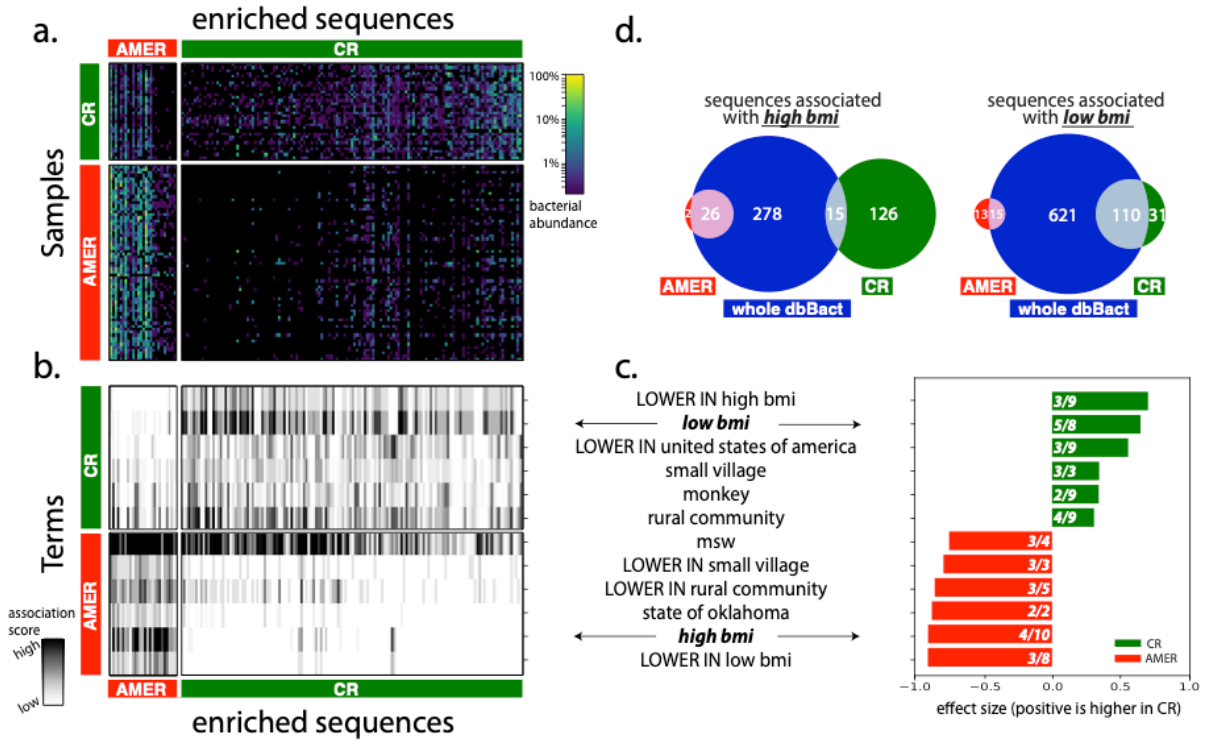


Figure 5. Linking caloric restriction associated bacteria to other phenotypes. a. Heatmap displaying bacterial abundances across fecal samples (rows) of low BMI individuals (BMI<25) practicing either a caloric restriction diet (CR, n=33) or an American diet (AMER, n=66), over a set of *sequences* (columns) that are significantly higher in either group. A differential abundance test (rank-mean test with dsFDR=0.1 multiple hypothesis correction) identified 136 bacteria higher in the CR group (S-CR) and 27 bacteria higher in the AMER group (S-AMER). **b**. dbBact *terms* (rows) enriched in the *sequences* appearing in panel **a** (columns in panels **a** and **b** are aligned). Heatmap values indicate the *term* score for each bacterium. *Terms* were identified using a non-parametric rank mean difference test with dsFDR=0.1 (top 6 terms for each direction are shown; see Supplementary File 4 for full list of enriched *terms*). **c**. Summary of the top enriched *terms* in the CR and AMER diets (green and red bars, respectively). Bar length and numbers are as in Figure 2. **d**. Venn diagrams of dbBact *annotations* related to the *terms* "low bmi" (right) and "high bmi" (left). Green and red circles indicate the number of *sequences* associated with the *term* in the CR and AMER diets, respectively; the blue circle indicates the number of such *sequences* across dbBact as a whole. The intersections of "low bmi" bacteria with the CR group are significantly higher (p=7E-5, using two-sided Fisher's exact test), confirming the association. Similarly, the intersection of "high bmi" annotated *sequences* across dbBact

with the AMER group is significantly higher than that with the CR group (p=3E-17, using two-sided Fisher's exact test).

dbBact allows formulating biological hypotheses

This section provides two examples of biological hypotheses based on the re-analysis of published data using dbBact. Two additional examples appear in Supplementary Results and are briefly described following the example given below.

Fruit consumption in American Gut participants

Samples from the American Gut project (50) were filtered based on levels of fruit consumption (higher/lower than three fruits per week). Stratifying for age, gender and BMI resulted in 1071 samples in each group (see Methods section "Datasets processing" for details). Standard analysis yielded 25 sequences in each group, referred to as S-high and S-low, respectively. dbBact enrichment analysis (Figure 6a) shows that S-low *sequences* were associated with diets in industrialized regions (e.g., "LOWER IN rural community"), and S-high was enriched with the *terms* "hunter gatherer," "monkey," and rural environment-related *terms*. For example, the *term* "monkey," appearing 5093 times across dbBact, is associated with 84% (21/25) of S-high *sequences*, compared to 16% (4/25) in S-low. This may indicate that the majority of bacteria positively affected by high fruit diet are universal responders to fruit consumption, and thus also appear in monkeys. Therefore, some of the differences in microbial communities between humans and monkeys are due to diet rather than host-microbe co-evolution.

Acute pancreatitis and Crohn's disease share gut bacteria

Fecal samples of 135 acute pancreatitis patients (seven days from the onset of symptoms) and of 35 healthy controls were analyzed (51). Standard analysis detected 39 *sequences* higher in pancreatitis patients and 300 *sequences* higher in healthy controls, referred to as S-panc and S-healthy, respectively. dbBact enrichment analysis (Figure 6b) shows that S-healthy *sequences* were enriched in health-associated *terms* such as "LOWER in crohn's disease" and "LOWER in ulcerative colitis," whereas enriched *terms* in S-panc *sequences* included disease associated *terms* such as "diarrhea" and "crohn's disease," as well as "LOWER in control." For example, 67% (26/39) of the S-panc *sequences* were independently annotated as "crohn's disease"-associated *sequences* across dbBact, compared to only 13% (40/300) of S-healthy. Therefore, dbBact hints at a common gut response to acute pancreatitis, diarrhea, and Crohn's disease, i.e., a phenomenon of general dysbiosis formerly suggested by Duvallet et al. (52). Next, we focused on the twelve

sequences in S-panc that are not associated with either Crohn's disease or ulcerative colitis across dbBact *annotations*. The *terms* associated with these thirteen *sequences* include "fermentation," "homo sapiens," "feces," and "skin," as well as "soil" and *sequences* tagged as "candidate contaminants" (Figure S3 and Supplementary File 5). Additional experimental validation is required to determine whether some of these bacteria are pancreatitis-specific.

Two additional examples are presented in the Supplementary results section. In the first example, dbBact unravels the sources of air samples in an urban region during a dust storm and during ambient air (53). dbBact found that fecal bacteria from human and various farm animals were present during ambient weather conditions, whereas dust storms brought bacteria associated with "desert" and "soil." The second example analyzes microbial profiling of the skin swabs of bank voles. Samples were collected inside the uninhabited Chernobyl exclusion zone, and compared to those collected outside the contaminated region (54). dbBact analysis indicated that some of the differences in skin microbial communities of bank voles inhabiting the two regions may be attributed to differences in exposure to humans and farm animals, rather than to exposure to radioactivity.

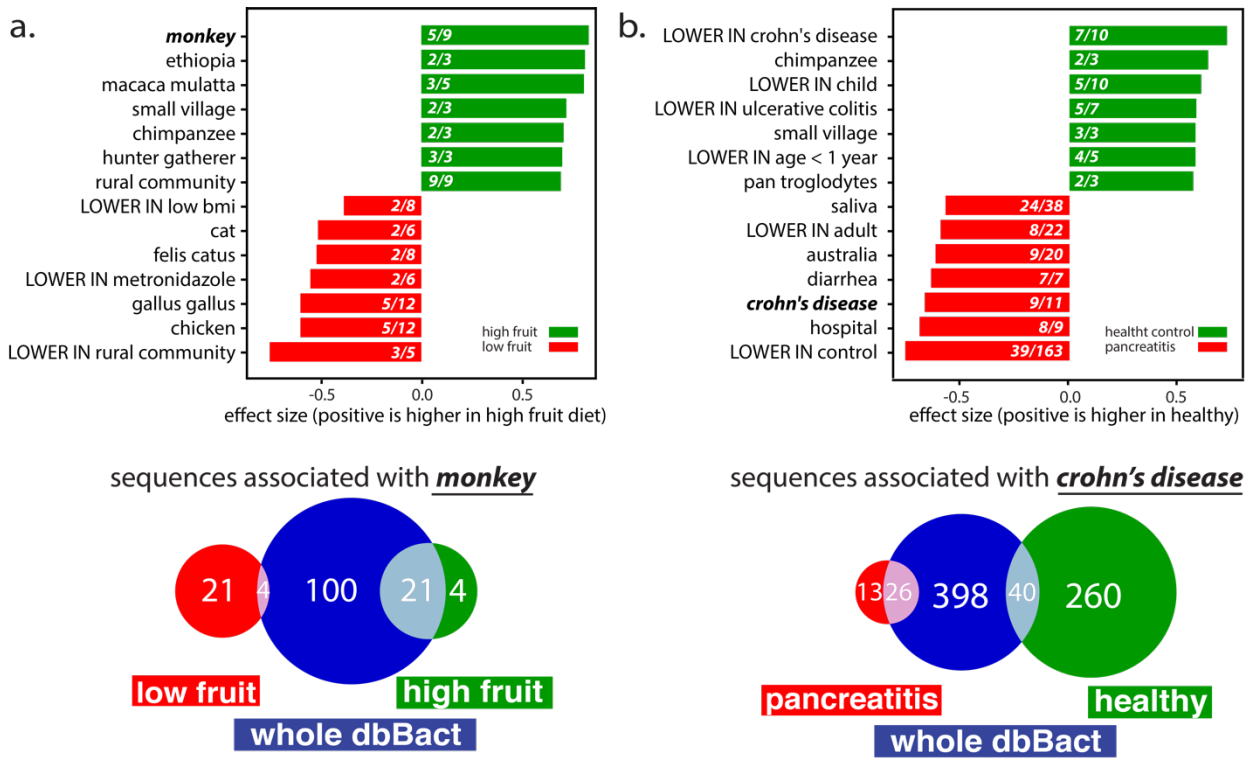


Fig 6. dbBact allows formulating biological hypotheses. Examples of pan-microbiome associations **a.** Fecal samples of American gut participants based on their fruit consumption. **b.** Fecal samples of acute pancreatitis vs. healthy controls. Panels display a bar plot of enriched *terms* in each group. Also shown is a Venn diagram for a representative *term* ("monkey" and "crohns" disease" in panels a and b, respectively), displaying the number of dbBact *sequences* associated with the *term* (blue circle), and their overlap with *sequences* associated with that *term* in either group (red and green circles). The numbers indicate the number of *sequences* in each part of the Venn diagrams.

Discussion

dbBact is a wiki-like bacterial knowledge base for collecting information from 16S rRNA amplicon studies. It creates a collaborative central repository suitable for subsequent analysis and it provides a pan-microbiome view.

An important advantage of dbBact, compared to standard meta-analysis methods, is that the latter may suffer from the "streetlight effect" (55). For example, when examining the effect of fruit consumption in the American Gut experiment (Fig 6a), one might consider including other diet-related studies in the meta-analysis. But this would miss the link between high fruit consumption

and primate-associated bacteria. dbBact retrieves *annotations* from a wide range of sample types and habitats, providing additional and potentially unexpected insights into the biological roles of bacteria.

Terms in dbBact *annotations* are based on ontologies, providing a common language for phenotype description. The tree structure of ontologies facilitates the discovery of commonalities between bacteria in studies conducted under similar, albeit not identical, conditions. For example, data from Crohn's disease and ulcerative colitis *experiments* may be combined based on their ontological "parent" *term* "inflammatory bowel disease." Moreover, many "cross-sectional" questions may be asked and possibly answered using dbBact. For instance, what *terms* are similar with respect to their bacteria (e.g., are dogs more similar to cats or to wolves?), or are there connections between phylogeny and specific phenotypes (e.g., does genus X appear only in host Y or in geographic location Z?).

Apart from putting forth novel hypotheses, dbBact makes possible the detection of sources of bacterial groups. We recommend querying dbBact as a first step in any microbiome analysis (e.g., using the interactive heatmap of the dbBact-Calour module). Identifying relevant bacterial groups and their dbBact *annotations* fosters an initial understanding of biological processes, supporting better downstream analysis. dbBact also enables associating bacteria with a "candidate reagent contaminant" *annotation*. We have encountered numerous cases where examining bacteria in a study detected contaminations, e.g., bacteria having mostly "mus musculus" *annotations,* although samples were of human origin.

The current coverage of dbBact is high in a large number of habitats, but many other habitats are still poorly covered (e.g., Figure 3e). Therefore, *terms* appearing in a small number of *annotations* may lead to dubious conclusions. For example, dbBact contains a single *experiment* originating from a scrubland environment. This *experiment* profiled the leaf microbiome of ivy plants, hence, querying a set of ivy leaf-related bacteria may result in the enrichment of both "ivy" and "scrubland" *terms*. Therefore, to avoid incorrect conclusions, users are advised to further examine the set of *experiments* associated with each enriched *term*. As the number and diversity of *experiments* in dbBact increase, such spurious *terms* are expected to be suppressed. Tens of

microbiome studies are published weekly, but the dbBact team can process only a fraction. We expect the microbiome community to contribute to dbBact and help increase the number and diversity of uploaded studies.

dbBact may also be used in shotgun metagenomics studies. Whenever 16S rRNA sequences are inferred from shotgun data they may be submitted as queries or uploaded to dbBact.
The "linking" mechanism for harmonizing studies from different variable regions enables shotgun and amplicon studies to be integrated into one coherent knowledge base. Similarly, studies using long read technologies (or synthetic long reads) also provide full-length 16S rRNA sequences, and thus can be integrated into dbBact in the same manner.

In sum, dbBact provides another layer of data analysis in microbiome studies, which we hope would be adopted by the community. Moreover, the scope and ontology-based structure of dbBact provides means for studying core factors affecting bacterial communities, possibly answering questions that could not have otherwise been asked.

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Methods

As dbBact is constantly growing in scope, and to facilitate reproducibility, the dbBact infrastructure described in this section and all analyses presented in the paper carried out using dbBact release 2021-05-09, available for download as part of the weekly snapshots at https://dbbact.org/download.

Implementation

Database

The dbBact database is stored as a SQL relational database (PostgreSQL 9.5.10). The database schema and detailed table descriptions are provided in Supplementary File 1 and Figures S5-6.

Ontologies

Table S1 presents ontologies available in dbBact release 2021-05. dbBact supports the addition of ontologies to allow more accurate annotations. When users provide *terms* that do not appear in any of these ontologies, a new *term* is automatically added to the generic dbBact ontology.

ASV sequences

Primers and trimming

dbBact uses exact prefix search for *sequence* identification, and therefore all *sequences* in dbBact are primer trimmed and originate from one of the supported 16S rRNA forward primers. For dbBact release 2021.05, the supported forward primers are V1-27F (AGAGTTTGATCMTGGCTCAGxxx), V3-341F (CCTACGGGNGGCWGCAGxxx), V4-515F (GTGCCAGCMGCCGCGGTAAxxx), where "xxx" denotes the beginning of the ASV sequence stored in dbBact. Although additional primers can be added to dbBact, the vast majority of 16S rRNA studies uses one of the three primers described. The minimum length of *sequences* uploaded to dbBact is 100bp. Upon upload, *sequences* are stored at their full length rather than being truncated to a fixed length. When submitting a query *sequence*, exact sequence matches are searched using *length=min(query_sequence_length, database_sequence_length)*.

Taxonomy assignment

A python script runs daily to add taxonomy assignments to uploaded dbBact ASV *sequences* using RDP version 2.12 (56). Although taxonomy is not used in dbBact for analysis, it may be used for querying dbBact (e.g., retrieving *annotations* associated with bacteria of the genus *Streptococcus*).

Inter-region querying

dbBact supports the harmonization of microbiome studies performed using different protocols by inter-region linking. When submitting a *sequence*, dbBact uses the SILVA database of full length 16S rRNA genes (SILVA version 132, (34)) to identify *sequences* whose "footprint" in other variable regions matches the query. First, the SILVA sequences containing the query *sequence* are detected. Second, all dbBact *sequences* that match these SILVA sequences, in any region, are retrieved (i.e., Query(S)={$T: T \in dbBact, \exists R \in SILVAsothatsubet(S, R) \wedge (T, R)$}). Querying is performed using the "wholeSeqIDsTable" table in the dbBact implementation. To enable fast queries, a daily script is run on new dbBact *sequences*, linking all *sequences* sharing a SILVA sequence. Such linking is performed only when querying dbBact, therefore new versions of SILVA or other full length 16S rRNA databases may be seamlessly applied. Currently, dbBact supports linking the V1, V3, and V4 forward primer reads; additional primers may be incorporated if needed.

Queries of different sequence length

Sequences uploaded to dbBact may vary in length depending on the sequencing platform and sequenced region. When adding new *annotations*, dbBact stores the full-length sequence of each ASV. For example, when two *experiments* provide information about the same bacterium using 150bp and 200bp reads, respectively, dbBact stores these *sequences* as separate entries and links each *annotation* to the corresponding *sequence*. Yet, when submitting a query using either *sequence,* dbBact retrieves *annotations* using exact match on the shortest common sequence, also retrieving *annotations* related to the other *sequence*.

dbBact interfaces

REST-API server

The dbBact REST-API server (http://api.dbbact.org) is implemented in Python 3.6, using Flask version 0.12/Gunicorn v19.9 to handle web queries, and psycopg2 version 2.7.1 for handling Postgres data queries. Full API documentation is available at http://api.dbbact.org/docs. Examples using the REST-API for querying are available at: https://github.com/amnona/dbbact-examples. The REST-API enables access to all dbBact functions. Querying dbBact or adding anonymous *annotations* does not require registration. Registration by username/password enables editing *annotations* submitted by the same user.

dbBact website

The dbBact website (http://dbbact.org) enables dbBact *annotation* retrieval based on ASVs, taxonomy, or ontology *terms*. Additionally, the website provides word-cloud generation and *term* enrichment analysis. The source code for the website as well as deployment instructions are available on the dbBact-website github page (https://github.com/amnona/dbbact-website).

dbBact-Calour interface

dbBact is integrated into the Calour microbiome analysis program (https://github.com/biocore/calour), using the dbBact-Calour module (https://github.com/amnona/dbbact-calour). Using this interface, users can both query dbBact regarding bacterial sequences, and add new *annotations.* The dbBact-Calour module provides dbBact *annotation* retrieval from the interactive Calour heatmap display, showing all *annotations* associated with the selected *sequence*. Additionally, the module enables GUI-based creation of new dbBact *annotations* for selected *sequences*, and performs *term* enrichment analysis and word cloud generation. A Jupyter notebook tutorial is available at http://biocore.github.io/calour/notebooks/microbiome_databases.html.

The module also works with EZCalour, the full GUI version of Calour, (https://github.com/amnona/EZCalour). A tutorial for dbBact enrichment analysis using EZCalour is available at: https://github.com/amnona/EZCalour/blob/master/using-ezcalour.pdf.

Qiime2 plugin

The q2-dbBact plugin (https://github.com/amnona/q2-dbbact) enables dbBact *annotation*-based analysis using the qiime2 framework (35). The interface provides *term* enrichment analysis for the output of various qiime2 differential abundance plugins (ANCOM (57), Songbird (58), ALDEx2 (59), DACOMP (60), or a rank-mean method). Additionally, the plugin supports dbBact *term* word cloud and interactive heatmap generation.

Data availability

Table 1 details the locations of different dbBact sites

	Location
dbBact website	http://dbbact.org
dbBact website source code	https://github.com/amnona/dbbact-website
dbBact REST-API server	api.dbbact.org
Documentation for the REST-API	api.dbbact.org/docs
Examples for using the REST-API interface	https://github.com/amnona/dbbact-examples
Source code for the REST-API server	https://github.com/amnona/dbbact-server
Jupyter notebooks for figures presented in the paper	https://github.com/amnona/dbbact-paper
Weekly dump of the complete dbBact database (excluding user details)	https://dbbact.org/download

Table 1: Locations of dbBact sites

Standard analysis: Default dbBact preprocessing of an *experiment*

Although dbBact is a wiki-style knowledge base, the vast majority of *annotations* in dbBact release 2021.05 was added by the dbBact team. Studies were selected from published microbiome papers, and *annotations* were added following the re-processing of the experimental data, using a "standard" manual analysis pipeline as follows:

The raw data of each scientific paper (i.e., per-sample FASTA files and corresponding metadata) were downloaded using the provided accession (e.g., by SRA/ENA accession or Qiita (21) study ID). When data or metadata were not available, the authors were contacted and provided the missing data directly. When primer sequences were part of the reads, they were removed using a custom script (https://github.com/amnona/GetData). Subsequently, the Deblur pipeline (15) was applied to the reads of each sample (Deblur script version 1.1.0, using default parameters, https://github.com/biocore/deblur), resulting in a denoised biom table.

This biom table, together with the per-sample metadata, were manually re-analyzed using Calour (36), to add *annotations* capturing biological conclusions arising from the study. Three types of predicates were sought:

(i) "DIFFERENTIAL:" To detect sets of *sequences* associated with relevant conditions (e.g., sick vs. healthy), *sequences* significantly enriched between two conditions were identified using a non-parametric permutation based rank-mean test, followed by multiple hypothesis correction using dsFDR (61) (usually set to 0.1). The test was performed by the calour.diff_abundance() function. Correlations with continuous metadata fields (e.g., BMI) were detected with a permutation-based Spearman test with dsFDR correction using calour.correlation(). In both cases, the set of *sequences* higher or lower in one condition than in the other were then annotated as "DIFFERENTIAL," i.e., "HIGHER IN" condition 1 and "LOWER IN" condition 2.

(ii) "COMMON"/"DOMINANT:" For each study, *sequences* present in more than half of the relevant samples were *annotated* as "COMMON." "DOMINANT" *sequences* were identified as *sequences* whose mean frequency in the relevant samples was higher than 0.01. In studies containing samples from multiple sources (i.e., fecal and saliva samples, or samples from individuals from several countries or disease vs. healthy), a "COMMON"/"DOMINANT" *annotation* was added separately to each source subset.

(iii) "CONTAMINANT:" When a study contained a set of negative control (blank) samples, ASVs showing higher frequency in these controls than in the non-blank samples were manually annotated as a possible "CONTAMINANT."

Examples of the different predicates appear in Table S3.

Remark: Using the abovementioned pipeline is not a prerequisite for adding new *annotations*, and any denoising method followed by statistical analyses can be applied by users contributing to dbBact.

Statistical analysis in dbBact

Word cloud generation

Calculating a term's F_1 *score*: Given a set of query *sequences* S, each dbBact *term* t is assigned an F_1 score, corresponding to the harmonic mean of precision and recall. For each sequence s in S, we calculate the the fraction of s's *annotations* that contain the *term* t. The average of these

values across S provides the precision of t on S. Similarly, for recall we calculate the fraction of t's *annotations* that is associated with each s. To suppress terms that appear in a small number of *experiments*, the total number of t's *annotations* (i.e., the denominator) is artificially increased by 1. The average of these values across S provides the recall of t on S.

Displaying a word cloud. The word cloud size of each *term* is proportional to its F_1 score. If the term appears in "LOWER IN" annotations, its color is orange, otherwise it is blue. The brightness of each *term* represents the number of *experiments* containing the *term,* indicating the reliability of the term (white for a single *experiment*; ranging to dark blue/orange for >=10 *experiments*). Word clouds were generated with the dbBact-Calour module for Calour (https://github.com/amnona/dbbact-calour) using the draw_wordcloud() function.

Term enrichment analysis

Given two sets of sequences, $S1$and $S2$, we search for *terms* significantly enriched in either group using the following steps:

(a) Calculating an annotation-based score per sequence and term. Each *annotation* a in dbBact is assigned a "weight" $w(a)$ according to its predicate. The predicates "COMMON," "CONTAMINATION," and "DIFFERENTIAL" are assigned a weight of 1, and the predicates "DOMINANT" and "OTHER" are assigned a weight of 2 and 0.5, respectively. These weights are applied to calculate a score $Score(s,t)$for each *term* t in dbBact and a *sequence* s in either $S1$or $S2$. The score sums the weights of all *annotations* involving s and t. When t appears as "LOWER IN" in the predicate "DIFFERENTIAL," a new *term* "not t" is created and assigned a weight of 1.

(b) Calculating effect size of a term. For a *term* t, $Score(s,t)$ is calculated for all *sequences* in $S1$and $S2$, and the effect size of t is defined as

$$e(t) = 2\left|\left(\frac{\sum_{s\in S1} Score(s,t)}{|S1|} - \frac{\sum_{s\in S2} Score(s,t)}{|S2|}\right)\right| \Big/ (|S1| + |S2|)$$

where $|S|$ corresponds to the number of *sequences* in the set S.

(c) Finding significant terms. Each *term* is assigned a p-value by comparing its scores over 1000 random permutations of the $S1$ and $S2$ *sequences* to sets of size $|S1|$ and $|S2|$. Subsequently, a dsFDR multiple hypothesis correction (with a threshold of 0.1) is applied to detect significant *terms*.

(d) Calculating the significance of a term across experiments. Until this stage, we measured the enrichment of a *term* based on all dbBact *experiments* combined. To estimate whether such significance occurs across multiple *experiments,* or whether it is driven by a single or a few *experiments*, steps (a)-(c) were also repeated using each individual *experiment* that contains t. The total number of *experiments* containing each *term* and the fraction in which the *term* was significant appear in each figure.

The abovementioned analysis is performed using the dbbact-calour enrichment() function.

Venn diagrams

Given two sets of sequences, $S1$ and $S2$, and a *term* t, we plot a Venn diagram indicating the number of *sequences* associated with t across all dbBact *annotations*, and the overlap of these *sequences* with $S1$ and $S2$.

Dataset processing

All datasets discussed in the paper were processed using the following pipeline: raw reads were downloaded and denoised using the Deblur pipeline (15) with default parameters; the resulting denoised biom table was loaded into Calour (36), and differentially abundant bacteria were identified using a permutation-based non-parametric rank mean test with dsFDR multiple hypothesis correction (61) set to 0.1 (using the calour diff_abundance() method). In the case of the American Gut Project dataset, multiple samples originating from the same individual were aggregated to a single sample using mean frequency for each ASV. The groups of high and low fruit consumption were controlled for confounders by stratifying samples in both groups based on the AGP metadata fields: age category ("AGE_CAT"), sex and BMI category ("BMI_CAT"), and randomly dropping samples to equalize the number of samples from each stratum prior to differential abundance testing. *Term* enrichment was performed using the Calour enrichment() method with default parameters (dsFDR=0.1) against dbBact release 2021.05. Accession numbers for each dataset used are available in Supplementary File 2. Jupyter notebooks used for the creation of each figure are available at https://github.com/amnona/dbbact-paper.

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