ADDENDUM

Cultured microbes represent a substantial fraction of the human and mouse gut microbiota

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**ABSTRACT**

During the last 15 years, molecular techniques have been preferred over culture-based approaches for the study of mammalian gut microbiota, i.e. the communities of microorganisms dwelling in the intestine of mammals. The main reason is the belief that the majority of gut bacteria, especially strict anaerobes, escape cultivation. Despite numerous such statements in publications, the literature does not provide a clear overview on the subject. In the present manuscript, we highlight recent work on the cultivation of bacteria from the intestine of mammals, review the literature and provide novel data pertaining to cultured fractions of mammalian gut microbiota. These data show that, despite marked inter-sample variations, 35 to 65\% of molecular species detected by sequencing have representative strains in culture.

**KEYWORDS**

16S rRNA gene; anaerobic cultivation; cultured fraction; dark matter; gut microbiota; intestinal bacteria; microbial diversity; minimal microbiome

**Introduction**

Microbial diversity on earth is tremendous, and functions performed by microorganisms are central to numerous essential bioprocesses, ranging from global events such as the cycles of elements (carbon, nitrogen, sulfur)\textsuperscript{1} and the large-scale production of food and drugs\textsuperscript{2,3} to microscopic interactions happening in our intestine.\textsuperscript{4} In gut environments, diverse and dynamic populations of bacteria interact constantly with dietary factors and with host cells and thereby influence many physiologic functions of the host such as metabolism and inflammation.\textsuperscript{5,6} To understand microbiome-diet-host interactions, it is important to characterize members of complex gut microbial communities. Despite over a century of research in microbiology, it is still challenging to accurately estimate the total prokaryotic diversity existing in all kinds of environments. Recent extrapolations based on sequencing data referred to approximately 400,000 species.\textsuperscript{7} Other estimates are considerably higher and range from $10^7$ to $10^9$ \textsuperscript{8} or even $10^{12}$ species.\textsuperscript{9} If we do not know exactly how many prokaryotes are there, it is of course difficult to define the fraction that can be cultured. However, it is certain that the unexplored or yet to be described diversity (also referred to as dark matter) is still substantial, leaving exciting research discoveries to come. Throughout the present manuscript, we review past and recent work on the cultivation of mammalian gut bacteria and provide an overview of cultured fractions of complex gut microbial communities.

**Culturing bacteria: An old quest, new opportunities**

The field of microbiology was driven at the beginning by the cultivation of microorganisms, especially pathogens. Although pioneers such as \'{E}lie Metchnikoff had recognized the potentially beneficial role of specific bacteria relatively early,\textsuperscript{10} the diversity of symbiotic gut bacteria and their implication for health and the development of diseases have received tremendous attention in recent years, thanks to breakthroughs in modern sequencing technologies.\textsuperscript{11} Previous to this molecular era, the study of intestinal bacteria had been boosted in the 1960s and 1970s by the emergence and utilization of anaerobic cultivation techniques, which gave access to many bacterial species that were not within reach beforehand.\textsuperscript{12,13} Researchers were at the time primarily interested in describing bacteria from the human gut and characterizing their diversity.

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and adaptation to variations in the diet or environment. The gut microbiota of animals was also an important topic of research at the time. Laboratory mice were studied to elucidate mechanisms underlying infections, leading to the development of standardized mouse models via colonization of germfree animals (kept in isolators in the absence of living microorganisms) with defined consortia of bacteria. Bacteria in the intestine of domestic animals were investigated for production purposes, i.e., to support animal growth or their well-being and to address environmental issues (e.g., production of methane).

Following the use of anaerobic cultivation in these pioneering years and throughout the 1990s, microbiology of the current century has been driven by high-throughput molecular methods that offered innovative approaches for the assessment of microbial diversity and functions. These methods are high-throughput, detect microorganisms missed by cultivation, and most of all allow integration of data at the level of entire ecosystems, revealing ecological principles that cannot be captured by the study of single organisms. Nevertheless, after 15 y of intensive research in the field, bottlenecks have also become obvious:

(i) Data analysis is bound to the quality of databases. A substantial part of the biologic information acquired by high-end sequencers or mass spectrometers is still to date not interpretable because of the lack of references.

(ii) Besides some innovative studies that bring new concepts to light, most of molecular investigations provide descriptive data, the interpretation of which remains very often speculative. In other words, molecular methods have limitations too, and generate many hypotheses that remain in most instances untested.

(iii) The broadened view of bacterial diversity gained by molecular methods revealed that many dominant and functionally important bacteria in various ecosystems still have no representative strains in culture. Until synthetic biology allows reliable and affordable reconstruction of native microorganisms and as long as the expression of native metagenomic functions is limited by cloning success and heterologous expression, the cultivation of native strains will remain the precondition for downstream functional studies of microbes and their interactions with mammalian hosts.

Hence, a renewed interest in cultivating mammalian gut bacteria exists nowadays. Contemporary cultivation studies are a great opportunity to utilize resources that our predecessors in the 1960s and 1970s did not have:

(i) Technological advances allow the development of innovative and/or large-scale isolation procedures that greatly enhance our chances to discover novel diversity and to increase the pool of cultured strains available.

(ii) Infrastructures for public archiving and dissemination of new strains have improved, even though seeking financial support for this fundamental aspect of the work remains a great challenge.

(iii) Most of all, being able nowadays to combine culture effort with molecular investigations is a major advantage: culture conditions of bacterial groups with particular ecological features can be inferred - at least in part - from genomic information; in turn the ecology and functions of novel isolates can be studied in great detail on a genetic level.

In the following section, we highlight new findings obtained by recent culture-based investigations of mammalian gut microbiota.

**New kids on the block**

The study of microbial populations in the rumen of cows has long been a driving force in the field of gut microbiology. It is thus not surprising that one main large-scale cultivation project of the last years originated from the initiative “Rumen Microbial Genomics Network” and aimed at cataloging genomes from rumen isolates, with particular focus on methanogenic archaea and hydrogen consumers. This project, referred to as the Hungate1000 project, eventually led to the isolation of approximately 600 taxa and the generation of nearly as many genomes now available from public databases.

The study of human gut microbiota has been of course a primary interest in recent years too. The research group of Didier Raoult in France has been very prolific in isolating bacteria from human feces by using a multitude of different agar media in combination with high-throughput identification of isolates by means of mass spectrometry, followed by genome
sequencing of interesting isolates. Another very recent work focusing on the study of human gut microbiota by means of cultivation was performed in the laboratory of Trevor Lawley in England. Besides discovering many novel species as in the other studies, (meta)genomic sequence analysis in the latter work led to the conclusion that approximately half of the bacterial genera found in a healthy gut microbiota have the potential to form spores, facilitating transmission between individuals. These projects demonstrate that hundreds of novel bacteria, taxa can still be discovered even by anaerobic cultivation on agar plates (the experiments were performed however at scales without precedence in the past). Nevertheless, these publications also highlight the challenge of describing all these isolates taxonomically in a rapid yet thorough manner necessary for proper implementation of public collections and databases. It is beyond scope of the present paper to address in details issues related to bacterial taxonomy. The field is currently experiencing necessary changes in paradigm to accommodate genome-based data for description of novel taxa. Nonetheless, rapid and non-validated naming of newly discovered bacteria generates confusion. Hence, there is urgent need to find a consensus on appropriate workflow and novel minimal standards required for describing new species.

One major advantage of cultivating bacteria is the ability to describe in detail their physiology and interactions with the host and with other members of the ecosystem by performing functional experiments. Key species in the human intestine have been identified by means of anaerobic cultivation. For instance, Akkermansia muciniphila is a dominant human gut bacterium that lives in proximity with the epithelium and is able to utilize mucin for growth. Its role in the development of metabolic disorders has been studied in detail and beneficial attributes have been associated with this species. Other bacteria of particular interest are butyrate producers, as this short-chain fatty acid is an important product of bacterial fermentation in the intestine, usually detected in the mM range and influencing many pathophysiological functions. The species Faecalibacterium prausnitzii is an important butyrate producer in the human gut and has been studied in great detail because of anti-inflammatory properties. Another butyrate producers more recently described is Intestinimonas butyriciproducens. Culture- and genome-based investigations elucidated the role of this bacterium in butyrate production not only from sugars but also from amino acids, a feature that is rare among human gut isolates. Interestingly, this species is found in both the human and mouse intestine, and differences in the ability to convert dietary sugars were observed depending on the host origin of strains. Other bacteria are known to preferentially colonize certain host species, and the origin of bacteria can influence physiologic functions upon colonization. This highlights the necessity to assess the gut microbiome in a host-specific manner. In the case of mice, many studies of the last 10 y had investigated their gut microbiota using sequencing techniques, and numerous research projects have relied on the use of germfree mice to assess mechanisms underlying microbe-host interactions. In contrast, culture-based investigations of the mouse gut microbiota had been rare in the last 2 decades and access to isolates has been a problem.

To address these issues, the mouse intestinal bacterial collection (miBC), a publically available repository of bacterial strains from the mouse intestine, was recently created (www.dsmz.de/miBC). As in the case of culture-based studies of the human gut microbiota, this work allowed the description of novel taxa, including for instance the first cultured member of family S24–7 (now renamed Muribaculaceae) within the Bacteroidales, which is highly prevalent and dominant in the mouse intestine. Of the 100 miBC strains representing 76 species, 19 species were characterized by relative abundances higher in mice than human, speaking in favor of colonization preferences. Most of all, strains in the collection can now be used for the design of model ecosystems that help understanding the role of gut bacteria in disease or supporting the development of standardized mouse models. Coverage of 16S rRNA gene amplicon data by the cultured strains was also assessed, revealing that numerous bacterial species from the mouse intestine remain to be discovered. In the next section, we review literature data pertaining to the cultured fractions of mammalian gut microbiota in greater detail.

**Did you say uncultured?**

As mentioned above, estimating total prokaryotic diversity on earth is very challenging, and estimates of global species richness vary greatly (10^5 to 10^12 different species). It is thus all the more challenging to appreciate the part of this diversity that can be maintained in culture. Here too the range of estimates is wide (from < 1 to > 90%), due of course to the type of
environments under investigation, but also to an overall lack of clarity regarding experimental approaches used and the parameters considered for calculating cultured fractions of complex communities. We provide below some reference values from the literature pertaining to cultured bacterial communities in the mammalian intestine.

First of all, it is good to remember that all microorganisms are potentially “cultivable.” The challenge is to overcome difficulties in finding appropriate conditions to isolate and grow many of them. Hence, the terms “cultured” or “not yet cultured” are more appropriate when referring to species already grown in laboratories or those for which appropriate conditions remain to be determined, respectively. Approximately 25,000 bacterial strains and the type strains of about 80% of all described bacterial species are archived to date at the German Collection of Microorganisms and Cell Cultures (DSMZ), one of the leading international public repositories of microorganisms together with e.g., the Japanese (JCM) and American (ATCC) collections. The latest version of the All-Species Living Tree in SILVA contains approximately 12,000 16S rRNA gene sequences from type strains, and databases such as BacDive gather precious phenotypic information of cultured diversity. These resources represent the total cultured diversity available to date, which leaves a substantial gap even to the lowest estimates of existing total bacterial diversity (10^5 species). In 2014, approximately 1,000 different bacteria isolated from the human intestinal tract were reported, and this figure has been increased by several hundreds of new species obtained thanks to recent effort in cultivating human gut commensals.

Moreover, initiatives describing novel bacterial diversity from other environments such as the mouse gut or plants can also help characterizing the human gut microbiota if the same bacterial species or very close relatives happen to colonize our intestine as well.

It is difficult to extrapolate the diversity of cultured bacteria as fractions of total diversity they represent. The first degree of complexity relates to the type of estimates under consideration: What is the reference used to express cultured fractions as percentages of a whole? Is it the percentage of total bacterial density countable under the microscope (with or without staining) or the total diversity as detected by sequencing? The second degree of complexity is that confusion can arise from the imprecision inherent to experimental techniques used to determine total bacterial density or diversity in target samples, not to mention that these samples should be representative and sufficient numbers thereof be analyzed. Recent reports drew attention to the spread of erroneous values regarding total bacterial cell density in the human gut or species richness detected by sequencing. Total counts determined by growing cells under only one culture condition have a very high risk of underestimating cultured fractions if this condition does not support growth of many species. Recent studies highlighted the usefulness of varying culture conditions to obtain novel bacterial diversity. Underestimation of cultured fractions may also be due to falsely high total cell densities due to the counting of debris. In contrast, cultured fractions may be overestimated if staining procedures are used to determine total densities, since staining can lead to loss of cells. Without aiming at an exhaustive list of studies, Table 1 provides some reference estimates of the cultured fraction of mammalian gut bacteria.

According to the experimental limitations mentioned in the previous paragraph, one obvious conclusion from the data presented in Table 1 is that the span of the cultured fraction of mammalian gut microbiota is unreasonably wide. Nevertheless, more than half of the listed studies based on various experimental approaches reported cultured fractions > 50%. Hence, when compared with the first paper coining the great plate count anomaly referring to cultured fractions < 1% in water environments, the situation in the mammalian gut may not be as bad as claimed by passionate supporters of molecular methods. In other words, it is in our opinion safer to say that approximately half of bacterial diversity in the human gut can be cultured, rather than a minor proportion. Moreover, a recent review by Walker et al. noted that cultured species are not evenly distributed along a gradient of dominance, i.e., most dominant species tend to be readily cultured whereas many of less dominant species remain to be characterized. Nevertheless, it must be acknowledged that the ever increasing numbers of cultured bacteria available originate from cumulative effort in cultivating microbes, and that single studies are per se limited in terms of width and depth of analysis.

To complement this literature review and test corresponding reference values of cultured diversity, we present in the next section original data pertaining to estimates obtained by large-scale 16S rRNA gene amplicon analysis.
**Universal molecular investigation toward the estimation of cultured bacterial diversity**

The cultured fraction of bacterial communities can be determined by measuring the number of 16S rRNA gene sequences obtained by high-throughput sequencing that can be assigned to cultured representatives. However, most studies performed so far relied on a limited number of datasets. For accurate estimation, 2 elements are important: (i) A complete reference set of full-length sequences from all known cultured bacteria; (ii) A large and representative number of samples and amplicon sequences from target environments.

(i) Because there is no constantly updated list of cultured species with corresponding 16S rRNA gene sequences easily available, we compiled such a resource from several databases by collecting all entries in NCBI taxonomy, SILVA (release 128), and LPSN (http://www.bacterio.net) with a binomial Latin name (genus and species) and a 16S rRNA gene sequence available. This resulted in 14,734 species with a full-length sequence from all known cultured bacteria.

### Table 1. Literature-based estimates of the cultured fraction of mammalian gut microbiota.

| Cultured fraction | Experimental information | Samples | Reference |
|-------------------|--------------------------|---------|-----------|
| 2.6% | Microscopic vs. plate counts (blood agar in anaerobic jars); $3.2 \times 10^{11}$ vs. $8.3 \times 10^{9}$ cell/g wet weight. | Human feces (n = 10) | 64 (van Houte & Gibbons, 1966) |
| 14.3% | DAPI vs. plate counts (blood agar); $2.7 \times 10^{11}$ vs. $3.9 \times 10^{10}$ cell/g wet weight; Cultured fraction was 36.5% relative to total microscopic counts ($1.1 \times 10^{11}$ cell/g). | Human feces (n = 10) | 65 (Langendijk et al. 1995) |
| 20.8% | DAPI vs. plate counts (BH with hemin and yeast extract); $1.1 \times 10^{15}$ vs. $2.2 \times 10^{11}$ cell/g dry weight; Cultured fraction was 31% relative to total FISH counts ($7.1 \times 10^{11}$ cell/g). | Human feces (n = 1) | 66 (Suau et al. 1999) |
| 21.5% | DAPI vs. plate counts (blood agar); $7.6 \times 10^{10}$ vs. $1.6 \times 10^{10}$ cell/g; Cultured fraction was 37.1% relative to total FISH counts ($4.4 \times 10^{10}$ cell/g). | Human feces (n = 8) | 67 (Tannock et al. 2000) |
| 23.5% | Coverage of OTUs from 16S rRNA gene amplicon libraries (sequences clustered at 97%), expressed as relative abundance of short reads covered at the genus level (match in the culture collection > 95% sequence identity). | Mouse cecum (n = 93) | 28 (Lagkouvardos et al. 2015) |
| 32.6% | Microscopic vs. plate counts (medium 10); $3.2 \times 10^{11}$ vs. $1.1 \times 10^{11}$ cell/g wet weight | Human feces (n = 3) | 69 (Hayashi et al. 2002) |
| 56.0% | Percentage of OTUs with a taxonomic assignment at the species level that was also identified in the corresponding cultured population (collection of single strains from the same donor); Coverage was 70% at the genus level. | Human feces (n = 8 samples from 2 donors) | 70 (Goodman et al. 2011) |
| 57.0% | Microscopic vs. plate counts (medium not specified); $6.6 \times 10^{10}$ cell/g dry matter | Human feces (n = 50) | 15 (Finegold et al. 1975) |
| 58.0% | Microscopic vs. plate counts (modified medium 10); $6.6 \times 10^{10}$ cell/g dry matter | Human feces (n = 1) | 71 (Wilson et al. 1996) |
| 63.2% | Microscopic vs. plate counts (RGCA in pre-reduced roll tubes); $4.1 \times 2.6 \times 10^{11}$ cell/g dry matter | Human feces (n = 6–9 samples from 3 donors) | 16 (Holdeman et al. 1976) |
| 65.0% | Microscopic vs. plate counts (RGCA medium); $1.1–1.5 \times 0.7–1.0 \times 10^{11}$ cell/g dry matter | Pig colonic content (n = 4) | 72 (Russell 1979) |
| 72.0% | Shared de novo assembled reads in shotgun metagenomes from fresh feces or the same samples cultured on YCFA agar. The proportion of shared metagenomics species was 73.5%, that of raw reads 93%. | Human feces (n = 6) | 35 (Browne et al. 2016) |
| 73.0% | Microscopic vs. plate counts (modified medium 10); $4.4 \times 3.2 \times 10^{10}$ cell/g wet weight | Mouse colonic content (n = 3) | 74 (Harris et al. 1976) |
| 93.9% | Microscopic (stained slides) vs. plate counts (RGCA in pre-reduced roll tubes); $5.1 \times 4.8 \times 10^{11}$ cell/g dry matter | Human feces (n = 20) | 75 (Moore & Holdeman, 1974) |
| 95.0% | Fraction of OTUs above 0.1% relative abundance with a corresponding species obtained by culturing using 66 different culture conditions | Human feces (n = 5) | 76 (Lau et al. 2016) |

Note. Gray studies and corresponding data refer to work based on next generation sequencing. Abbreviations: BH, brain-heart-infusion; DAPI, 4',6-diamidin-2-phenylindol; FISH, fluorescence in situ hybridization; NA, not available; OTU, operational taxonomic unit; RGCA, rumen fluid-glucose-cellobiose agar; YCFA, yeast-casitone-fatty acids medium.
sequence accession (corresponding to the type strain whenever possible), unique NCBI TaxID, and scientific name. Although more species with described isolates exist, they either have no sequence available or no name effectively published. Of note, bacterial names did not necessarily have to be valid in nomenclature to be included in our list (although most did), because we considered that a published bacterium, for which a 16S rRNA gene sequence is available, was isolated at least once and thus belongs to the pool of taxa that were proven to be cultured. We used the assembled 16S rRNA sequences list to build a local BLAST\textsuperscript{58} database of cultured bacteria.

(ii) To establish a comprehensive catalog of culture-independent, sequence-based diversity, we relied on the accumulated data available in IMNGS (build 1612),\textsuperscript{59} a web platform that automatically collects and utilizes 16S rRNA amplicon sequences from public repositories, currently hosting over 88,000 profiles of samples processed de novo into OTU tables. We filtered the complete list of samples from human and mouse gut resulting in 16,667 and 9,369 preprocessed sample profiles, respectively. For each sample, we extracted the OTU table and stored information on the richness of OTUs, their relative abundance, and their sequences.

To calculate cultured fractions, we performed a BLAST similarity search of every OTU sequence in each IMNGS-derived human and mouse gut sample against our local database of 16S rRNA sequences from cultured taxa. An OTU was considered to match a cultured taxon at the species or genus level if sequence similarity was \( \geq 97 \) or \( \geq 95 \)%, respectively, for at least 80% of the amplicon length. Since many of the OTUs from the long tail of low relative abundances have a very high risk of being artifacts, we performed the analysis twice: once with all OTUs, once with an extra filter removing OTUs with a relative abundance < 0.5% total sequences in the given sample. The fractions of cultured taxa in the metagenomic data sets were expressed either as proportions of the number of OTUs or their cumulative relative abundances.

![Figure 1](image-url)

**Figure 1.** Estimates of the cultured fraction of human and mouse gut bacteria and archaea as detected by high-throughput sequencing of 16S rRNA amplicons. The analysis was based on 14,734 full-length sequences of isolates and 26,036 complex gut microbial profiles (16,667 from human and 9,369 from mouse). Single OTUs from these profiles were matched to the isolate sequences, and those with similarities above the conservative species- (97%) and genus- (95%) specific levels were considered to originate from cultured bacteria. Results were expressed either as percentage of OTU richness or corresponding cumulative relative abundance of sequences. The figure illustrates data obtained when considering OTUs that occurred at a relative abundance > 0.5%. For estimates based on all OTUs, readers are referred to Supplemental Figure S1.
This analysis, performed at a scale never reached so far, provided several outputs summarized in Figure 1 and Supplemental Figure S1:

(i) Independent of the host (human or mouse), median estimates of cultured fractions at the species level were >20% in terms of OTU numbers and > 40% in terms or cumulative relative abundance of sequences. At the genus level, values were ≥ 50%.

(ii) In agreement with the primary focus of past cultivation efforts, cultured fractions of human gut microbiota were overall 20 to 30% higher than for mice. This shows the importance of analyzing gut microbiota in various host species to obtain a comprehensive view of mammalian gut bacterial diversity.

(iii) When limiting the analysis to dominant OTUs (those occurring at a relative abundance > 0.5%), we observed an increase of 10 to 25% of cultured fractions based on OTU numbers. In contrast, fractions based on cumulative relative abundances were logically not changed substantially, as less dominant OTUs represent per definition a minor proportion of total sequences. This increase in cultured fraction after OTU filtering is related to the exclusion of artifact OTUs that do not occur in native ecosystems, but also to the fact culture approaches may perform relatively well in capturing dominant species. Nevertheless, it is important to remember that less dominant bacteria as detected in feces may carry important and singular metabolic functions, they may be the foundation of trophic chains involving dominant members, or may be dominant in specific niches. Hence, they should not be neglected.

Of note, marked inter-sample differences were observed, with a range of percentages spanning the entire scale from 0 to 100, albeit with gradients of samples density at different percentages of cultured fraction, as nicely shown by the violin plots. One limitation of our approach is the poor description of samples in databases. Hence, in the case of human samples, extreme values may correspond to individuals with a disease linked to substantial shifts in gut microbiota structure or to subjects taking antibiotics. In the case of mice, extreme values may also correspond to gnotobionts associated with microbiota from different environments or with minimal consortia of cultured strains. Moreover, analysis of short reads, even from variable regions of the 16S rRNA gene, limits the resolution of analysis and we cannot exclude that reads matching the sequence of cultured bacteria at the selected thresholds of similarity would still do across full-length sequences. Nevertheless, considering the breadth of analysis with thousands of samples, we consider that these novel data provide the most comprehensive overview of knowledge based on high-throughput sequencing available to date.

**Conclusion**

It is difficult to appreciate the amount of effort still required for obtaining a more exhaustive and accurate view of cultured microbial communities in the mammalian gut, mostly because the total diversity of native ecosystems is still challenging to assess, approaches at hand to estimate the proportion of native ecosystems can vary, and proper long-term handling and characterization of isolates is tedious. Furthermore, spatial distribution of microbial communities along the intestine is an additional challenge: some niches colonized by specific bacteria will usually be missed by classical investigations based on fecal material. Nevertheless, via the current renewed interest in isolating prokaryotes from the intestine of mammals, research is on a good track. The present review of literature is not exhaustive, but the studies mentioned combined with the new original data provided clearly show that, although there is no clear consensus in results, the situation is not as bad as claimed in the last decade: 50% of human gut prokaryotes being cultured is a very rough estimate that is more reasonable than previous statements referring to minor cultured fractions. It is also evident that additional effort is required to obtain a comprehensive view of intestinal microbial communities, and combining innovative culture techniques with molecular tools will be crucial. Thereby, a very important aspect of prokaryotic life in native environments that will have to be addressed is diversity and plasticity of the ecosystem at the strain level. It is well-known than different strains can have very distinct phenotypes and rapid exchange of genetic information creates important functional dynamics in native ecosystems. It remains to be proven that strain variants as detected by high-throughput sequencing are indeed associated with relevant functional changes. Moreover, the current gap in
cultured prokaryotic diversity at the level of species is already a challenging task and creating collections with the aim of capturing as many species as possible is a good starting point. Nevertheless, future work should include the extension of archives to multiple strains per species to allow further use of these strains in downstream experiments to investigate functional plasticity and evolution. Most importantly, the knowledge generated by contemporary culture-based research is not necessarily novel: past isolation campaigns 50 y ago had already led to breakthroughs in the understanding of cultured diversity, but strains and corresponding biologic knowledge have been lost over the years. Hence, it is crucial to support initiatives that aim at archiving bacterial diversity in the form of host-specific collections and well-curated databases.28,29,63

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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