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Culturomic recovery of the banana (Musa spp.) core microbiome creates a biobank for functional microbiome engineering

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Article

Keywords: core microbiome, culturomics, culture collection, plant growth promotion, biological control, Fusarium oxysporum f. sp. cubense

Posted Date: October 8th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10634569/v1

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Additional Declarations: No competing interests reported.

Abstract

Persistent core microbiome members are attractive targets for microbiome engineering because they are more likely than transient taxa to colonise hosts consistently across environments. However, most crop core microbiomes remain defined only by sequence data, limiting mechanistic understanding and practical deployment. Here, we used targeted high-throughput culturomics to recover the previously defined Australian banana (*Musa* spp.) core bacteriome from field-grown plants. Across five isolation experiments, we obtained cultured representatives of 24 of the 36 core bacterial taxa, including much of the dominant fraction of the banana core microbiome. We established a biobank of 62 verified core isolates and selected genome-sequenced representatives of each of the 24 recovered taxa for functional characterisation. Genome annotation revealed broad genetic potential for plant-beneficial functions, including nutrient acquisition, phytohormone modulation, and antagonism of plant pathogens. Several representative isolates inhibited the banana pathogen *Fusarium oxysporum* f. sp. *cubense* (*Foc*) *in vitro*, and two significantly reduced *Foc*-induced rhizome necrosis *in planta*. Our study converts a sequence-defined crop core microbiome into a cultured and genomically characterised resource, providing a foundation for functional interrogation and the development of microbiome-based approaches to improve banana performance and resilience.

1. Introduction

Plants harbour diverse microbial communities that influence host nutrition, development, stress tolerance, and disease resistance, making the microbiome an important target for sustainable crop improvement[1,2]. However, translating microbiome knowledge into practical agricultural strategies remains difficult because plant-associated microbial communities vary across space and time. Taxa identified as beneficial in one location or production system may not persist or provide the same functions elsewhere, while introduced microorganisms may fail to establish within resident communities. This context dependence is a major obstacle to robust microbiome-based agriculture[3].

One way to address this challenge is to prioritise microorganisms that are consistently associated with a host under contrasting environmental conditions. In banana, Birt et al.[4] defined a common core microbiome comprising bacterial taxa shared across soil types, plant compartments, and host genotypes. These taxa were not only recurrent but also represented a substantial fraction of total community abundance in field-grown plants, occurred across multiple plant compartments, and were well represented in independent studies of banana-associated bacteria worldwide[5]. Thus, the banana core microbiome is not simply a collection of recurrent rare taxa but includes bacterial populations that are both persistent and quantitatively important components of the *Musa* microbiome. These properties make them logical targets for understanding plant–microbe interactions and developing microbiome-based strategies to improve crop performance.

Banana (*Musa* spp.) is one of the world's most important fruit crops and a major source of food and income in tropical and subtropical regions, yet its production is constrained by multiple abiotic and biotic stressors, including Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*)[6]. Microbial inoculants and biocontrol agents have shown promise for improving banana growth and suppressing disease, and several studies have isolated endophytes, rhizosphere bacteria, or taxa associated with healthy plants and screened them for beneficial traits *in vitro* and *in planta*[7–9]. However, candidate microorganisms have typically been identified without explicitly considering whether they are persistent members of the banana microbiome across contrasting environments. Consequently, their broader ecological relevance, including their capacity to persist across banana production systems and function alongside resident microbiota under differing conditions, remains uncertain.

The sequence-based identification of a common banana core microbiome provides a more hypothesis-driven route for prioritising ecologically relevant targets for isolation. Because the 36 core taxa identified by Birt et al.[4] were shared across soil types, plant compartments, and host genotypes, and represented a substantial fraction of total sequence abundance, they are likely to include bacterial populations well adapted to association with banana and able to persist despite edaphic and host-related variation. Building a culture collection of these organisms would therefore provide a valuable resource for mechanistic studies of plant–microbe interactions, for experimentally investigating the functions of core microbiome members, and for developing strategies to leverage beneficial taxa through inoculation, enrichment, or environmental management.

Despite major advances in sequencing-based microbiome research, obtaining targeted isolates remains a longstanding bottleneck. Traditional cultivation methods typically recover only a fraction of the bacterial diversity present in environmental samples[10–14], although recovery can be higher for plant-associated bacteria[15–17],, perhaps because plant-associated habitats are enriched in relatively simple substrates that are also common in laboratory media[18,19]. Conventional plating nevertheless favours organisms that grow readily under standard laboratory conditions and may therefore fail to recover specific taxa identified as ecologically relevant through sequence-based studies.

When the aim is to recover specific microbial targets rather than a random cultivable subset, cultivation success can be improved by combining prior ecological knowledge with highly parallel cultivation and sequence-based screening. For example, Bai et al.[20] used 16S rRNA gene amplicon sequencing to identify dominant bacterial taxa associated with *Arabidopsis thaliana* and then applied multiple cultivation strategies to recover representatives of those targets, including traditional plating, fluorescence-activated cell sorting, and large numbers of parallel liquid cultures followed by amplicon-based taxonomic screening. Together, these approaches recovered substantial proportions of the dominant root- and leaf-associated bacterial taxa, with highly parallelised liquid cultivation performing particularly well. More recently, high-throughput limiting dilution (HLD) culturomics coupled with amplicon-based screening has provided an efficient approach for recovering targeted bacteria from complex plant-associated communities[21].

Here, we used targeted culturomics to recover members of the banana core bacteriome defined by Birt et al.[4] and to establish a biobank of cultured core isolates. We combined an HLD culturomics workflow based on amplicon sequencing of highly parallelised cultures[21] with traditional plating on selective media, targeting the 36 core bacterial taxa previously identified in field-grown banana. We then used genome sequencing to verify correspondence with the

target core OTUs and infer functions associated with plant growth promotion and plant–microbe interactions. To determine how well the recovered taxa represented the dominant fraction of the banana core microbiome, we assessed their prevalence and relative abundance in bacterial communities from field-grown banana plants. Finally, we screened representative isolates for antagonism against *Foc in vitro* and evaluated the most promising strains for suppression of *Foc*-associated rhizome necrosis in banana plant. Together, this work converts a sequence-defined crop core microbiome into a cultured and genomically characterised resource for functional interrogation and microbiome-based crop improvement.

2. Materials and methods

2.1 Collection of banana root and leaf samples

Root (apical and basal) and leaf (newly emerged and mature) samples were collected in triplicate from *Musa* (AAA Group, Cavendish Subgroup) 'Williams' plants grown in a plantation established on Innisfail series soil[22–24] at the Centre for Wet Tropics Agriculture, South Johnstone, Queensland (17°36'12" S, 145°59'55" E). Samples were collected in sterile 50 mL tubes (roots) and zip lock bags (leaves) using sterile tools and gloves. Five isolation experiments were conducted over a two-year period (Fig. 1). On each occasion, field samples were placed immediately in a cooler containing ice packs, transported to The University of Queensland, and processed for cultivation within 24–36 h.

2.2 Isolation experiments

For each of the five isolation experiments (Fig. 1), fresh tissue samples were processed using either high-throughput limiting dilution (HLD) culturomics or traditional plating (Fig. 1). Across all experiments, a total of 38,880 HLD wells and 132 agar plates were inoculated with diluted tissue extracts (Fig. 1). A range of cultivation media was used for both approaches.

For HLD, we used the general media one-tenth-strength tryptic soy broth (TSB; roots only) and Reasoner's 2A broth (R2B; roots and leaves), as these media have been shown to support growth of most target lineages[20,25] (Figs. 1; Table S2; Supplementary Methods). For traditional plating, we used the general media Reasoner's 2A agar (R2A) and Czapek Dox agar (CDA) to recover a broad range of taxa from leaves, together with five selective media targeting specific bacterial groups where relevant (roots, leaves, or both) (Fig. 1; Table S2; Supplementary Methods): i) methanol mineral salts agar (MMSA) for *Methylobacterium* spp.[26], ii) mineral medium agar (MMA; pH 7.25) for *Sphingomonas* spp.[25], iii) glucose yeast malt agar (GYM) for *Streptomyces* spp.[25], iv) allantoin agar (AA) for *Bacillus* spp.[25], and v) Enterobacter agar (EA) for *Enterobacter* spp.[25]. Detailed media compositions are provided in the Supplementary Methods.

2.2.1 HLD-mediated isolation, purification, and temporary cryostorage

The HLD method was adapted from Zhang et al.[21] and is described in detail in the Supplementary Methods. Briefly, tissue extracts were aliquoted into 96-well plates containing general media at a dilution predetermined to yield visible bacterial growth in fewer than 50% of wells (Supplementary Methods, Table S1). According to Zhang et al.[21], this proportion balances isolate purity against community coverage. Plates were incubated at 24°C for 14 days with shaking at 20 rpm. From wells showing visible growth, 10 µL culture was transferred for DNA extraction, and 140 µL culture was mixed with 140 µL of 80% glycerol and stored at − 80°C.

2.2.2 Traditional plating-mediated isolation, purification and temporary cryostorage

For traditional plating, diluted tissue extracts were spread onto a range of general and selective media (Fig. 1, Supplementary Methods). Individual colonies were picked using a sterile loop, purified by repeated streaking, and transferred to the corresponding liquid medium. After incubation for 16 h at 25°C with shaking at 160–180 rpm, 10 µL culture was transferred for DNA extraction and 500 µL culture was mixed with 500 µL of 50% glycerol and stored at − 80°C.

2.3 Identification of putative core isolates

Detailed methods for the identification of putative core isolates are provided in the Supplementary Methods. Briefly, DNA was extracted using the alkaline lysis method described by Zhang et al.[21]. The 16S rRNA gene was amplified using modified 799F and 1193R primers as described by Birt et al.[4]. Amplicons were then dual-indexed using the Nextera XT Index Kit (Illumina) and sequenced on an Illumina MiSeq platform. Sequence data were quality filtered and clustered into 97% operational taxonomic units (OTUs), which were assigned SILVA v138 SSU taxonomy[27] to maintain consistency with Birt et al.[4]. OTUs were considered close matches to a core OTU if they shared ≥ 97% sequence similarity across 100% of the amplicon region with one of the 36 representative sequences of the banana core bacterial OTUs in a custom reference database derived from Birt et al.[4] (NCBI SRA accession: PRJNA72916a).

2.4 Final isolation and biobanking

Aliquots from cryopreserved HLD wells or isolate samples corresponding to OTUs classified as close matches to the banana core OTUs of Birt et al.[4] were streaked onto agar prepared with the same media used for the initial isolation and incubated at 25°C for 3–10 days. Single colonies were picked and purified through successive rounds of plating on the same medium. This yielded 90 pure isolates from HLD wells and six from traditional plating (Fig. 1, "CFUs picked for isolation and genome sequencing"). Pure isolates were then grown in 15 mL liquid medium for 16 h at 25°C with shaking at 160–180 rpm. For each isolate, 500 µL culture was mixed with 500 µL of 50% glycerol and stored at − 80°C for biobanking, while 5 mL culture was reserved for genomic DNA extraction.

2.5 Genome sequencing of putative core isolates

Detailed methods for genome sequencing are provided in the Supplementary Methods. Genomic DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) method[28]. Shotgun libraries were prepared for all putative core isolates, comprising the 90 HLD-derived isolates and six isolates recovered by traditional plating, as described previously[29], and sequenced on an Illumina NovaSeq platform. Long-read libraries were prepared using Oxford Nanopore Technologies kits and protocols (SQK-LSK109, EXP-NBD104, and EXP-NBD114) according to the manufacturer's instructions and sequenced on an R9.4.1 flow cell.

2.6 Processing of genome sequences and target verification

Details of the bioinformatic workflows used to generate genome assemblies from short- and long-read data are provided in the Supplementary Methods. To verify matches between genome-sequenced isolates and the core bacterial OTUs defined by Birt et al.[4], 16S rRNA gene sequences were extracted from isolate genomes using Prokka[30] (v1.13.3). These sequences were then queried against a custom reference database containing the 36 representative sequences of the banana core OTUs identified by Birt et al.[4] using USEARCH[31] (v11). Isolates were considered close matches, and assigned core isolate status, if their 16S rRNA gene sequence shared ≥ 97% similarity across 100% of its length with a representative core OTU sequence. Isolate genomes were also assigned GTDB r214 taxonomy[32] using the GTDB-Tk v2.3.2 *de novo* workflow[33].

2.7 Selection of representative 'core' isolates

All isolate genomes with a 16S rRNA gene matching a banana core OTU from Birt et al.[4] were dereplicated using dRep[34] (v2.6.2). Where a core OTU was represented by a single isolate, that isolate was selected automatically as the representative. Where multiple isolates matched the same core OTU, the representative isolate was chosen based on: i) average nucleotide identity (ANI) among genomes, calculated using fastANI[35] (v1.33); ii) genome completeness and contamination scores estimated with CheckM2[36] (v1.0.2); and iii) similarity of the genome-derived 16S rRNA gene sequence to the corresponding core OTU sequence from Birt et al.[4]. Representative isolates were therefore selected to maximise genome quality while remaining highly similar to the target core OTU and broadly representative of other isolates within the same group.

2.8 Core isolates and their representation in field-grown bananas

We next assessed the representation of the 24 cultivated core OTUs within our previously published dataset describing the ectorhizosphere, endorhizosphere, pseudostem, and leaf-associated microbiomes of field-grown banana plants[4]. First, we determined how many of the 'key constituents' of field-grown banana plants, defined by Birt et al.[4] as OTUs occurring in ≥ 50% of plants at ≥ 0.5% relative abundance, were represented by the 24 core OTUs for which isolates were obtained. Second, we calculated the percentage of total reads within each compartment (ectorhizosphere, endorhizosphere, pseudostem, and leaf) represented by these 24 cultivated core OTUs. Finally, we ranked the 36 banana core OTUs identified by Birt et al.[4] according to mean relative abundance within each plant compartment to determine whether dominant core taxa had been recovered by cultivation.

2.9 Genome annotation

For each genome, we summarised genome size, contig number, N50, and GC content using seqstats[37] and the stats.sh script from BBMap[38]. Genomes were annotated with Prokka[30] (v1.13.3) to identify coding sequences and the positions of rRNA, tRNA, non-coding RNA, and signal peptide genes. KofamScan[39] (v1.3.0) was then used to identify genes associated with nutrient cycling, plant growth promotion, and antagonism-related traits, including nitrogen fixation (*nifKHD*), phosphorus solubilisation (*gcd*), thiosulfate oxidation (*tsdA*, *soxABCDXYZ*), amylase production (*AMY*, *treS*, *cd*), ACC deaminase (*acdS*), cytokinin biosynthesis (*miaA*, *log*, *miaB*, *miaE*, *CKX*, *tzs*), β-1,3-glucanase production (*bglX*, *EBG*), zinc uptake (*znuABC*, *troBCD*), indole-3-acetic acid production (*aofH*, *iaaM*, *ipdC*, *tyrB*, *nthA*, *nthB*, *tdc*, *amiE*, *nit*), and chitinase production (*chiA*). We also used DRAM[40] to identify genes associated with carbohydrate-active enzymes (CAZy), including those linked to amorphous cellulose degradation, and pectinases annotated via MEROPS. Finally, siderophore and terpene biosynthetic gene clusters (BGCs) were identified using antiSMASH[41] (v6.0.1). Complete outputs from the KofamScan, DRAM, and antiSMASH analyses are provided as Supplementary Data.

2.10 *In vitro screening of representative core isolates for inhibition of Foc*

The 24 representative core isolates were screened for inhibition of *Foc* Race 1 (*Foc*-R1; BRIP43996) and *Foc* subtropical race (FocSR4; BRIP40272) using a dual culture assay on quarter-strength potato dextrose agar (PDA), following Batista et al.[42]. Percentage inhibition of fungal growth was calculated as:

$$I\,(\%) = \frac{D_C - D_T}{D_C} \times\ 100$$

where $D_C$is the diameter of fungal growth in the absence of the bacterial isolate and $D_T$is the diameter of fungal growth in the presence of the bacterial isolate.

2.11 *In planta screening of selected representative core isolates for suppression of Fusarium wilt*

Selection of isolates and experimental setup

The four representative core isolates that showed the strongest inhibition of *Foc*-R1 and *Foc*-SR4 *in vitro* (8, 10, 42, and 61) were selected for *in planta* screening of *Foc*-R1 disease suppression in banana plants (Supplementary Methods). The experiment was conducted from September 2022 to February 2023 at the Centre for Wet Tropics Agriculture, South Johnstone, Queensland, Australia (17°36'12" S, 145°59'55" E), using Dwarf Ducasse banana plants (ABB). Banana plantlets were sourced from Yuruga Nursery, Walkamin, Queensland, Australia. Six-week-old banana plantlets, grown in Innisfail series soil

collected from a non-banana site, were inoculated with either a representative core isolate or a water mock treatment on days 0 and 20. At day 54, half of the plants in each treatment were inoculated with *Foc*-R1 by placing *Foc*-R1-infected banana pseudostem on the soil surface around each plant. Infection pressure was reinforced at day 72 by applying a cell suspension of *Foc*-R1 (; 4.45×10^6 spores pot^{-1}) to all *Foc*-R1-inoculated plants. An equal volume of sterile water was applied to uninoculated plants. At the start of the experiment, eight replicate plants were established for each *Foc*-R1-inoculated treatment and four for each uninoculated treatment.

Assessment of disease severity: At day 169, external and internal disease ratings were recorded. To quantify internal necrosis, four cross-sections were taken from each rhizome: (i) one-quarter of the distance from the base of the rhizome, (ii) halfway through the rhizome, (iii) three-quarters of the distance from the base, and (iv) at the junction between the rhizome and pseudostem (Fig. 8). Images of these cross-sections were analysed in ImageJ[43] to quantify tissue discolouration caused by Fusarium wilt using the method described by Orr et al.[44]. Percentage necrosis was calculated as:

$$\text{Necrosis} = 100 - \left(\frac{\text{Mean}}{\text{Max}} \times 100 \right)$$

where *Mean* and *Max* are values returned by the ImageJ Analyse and Measure function.

Analysis of necrosis data

Rhizome necrosis data were analysed to determine whether inoculation with each isolate significantly reduced this key symptom of Fusarium wilt in banana plants. Necrosis values were $\log_2$-transformed as $\log_2$ (necrosis + 1). The effect of treatment on rhizome necrosis in *Foc*-R1-inoculated plants was assessed using a generalised linear model (GLM) with a Gaussian error distribution. Pairwise comparisons among treatments were performed using least-squares means predicted from the GLM, as implemented in lsmeans[45].

3. Results

3.1 Composition of the culturomes

Of the approximately 40,000 culture vessels (wells or plates) inoculated with extracts from banana roots and leaves, 4,058 showed visible microbial growth and were subjected to 16S rRNA gene metabarcoding (Fig. 1). Across these cultures, 411 OTUs were identified, of which 356 (86.6%) were bacterial. These bacterial OTUs comprised 183 Proteobacteria, 72 Actinobacteriota, 50 Firmicutes, 30 Bacteroidota, 8 Chloroflexi, 6 Acidobacteriota, 2 Deinococcota, 2 Verrucomicrobiota, 1 Bdellovibrionota, 1 Nitrospirota, and 1 Planctomycetota (Fig. 2; Table S3).

Of the 356 bacterial OTUs recovered across all isolation experiments, 99.2% were detected using HLD culturomics, whereas 25.0% were detected using traditional plating across seven agar media (Fig. 1; Table S3). Cultivation method was associated with a statistically significant but small difference in phylum-level composition (PERMANOVA on the OTU table summarised at phylum level: R^2 = 1.29%, F = 52.874, P < 0.001). Thus, despite the substantially greater number of bacterial OTUs recovered through HLD, the two approaches produced broadly similar proportional representation of major bacterial phyla.

3.2 Biobanking of isolates corresponding to core OTUs

Of the 356 bacterial OTUs recovered in the culturomes, 29 shared ≥ 97% sequence similarity with 28 of the 36 banana core OTUs identified by Birt et al.[4] (77.8%; Figs. 1, 2). Notably, these 29 OTUs were highly represented within the culturomes, collectively accounting for ≥ 50% of reads in approximately 41% (1,660/4,058) of all cultures analysed. A further 60 culturome OTUs were assigned the same SILVA v138 SSU taxonomy as one or more core OTUs from Birt et al.[4], but shared < 97% sequence similarity and were therefore not considered sufficiently close matches for further investigation (Fig. 2).

We next revived 78 cryopreserved cultures containing the 29 OTUs that matched 28 of the 36 core OTUs identified by Birt et al.[4] (Fig. 1). From these cultures, 96 colony-forming units (CFUs) were recovered, purified, and subjected to genome sequencing (Fig. 1). Following genome quality assessment, 15 isolates were discarded, leaving 81 genomes for downstream analyses (Fig. 1). Of these, 62 isolates harboured 16S rRNA genes sharing ≥ 97% sequence similarity with 24 of the 36 banana core OTUs identified by Birt et al.[4], equivalent to recovery of 66.7% of the core bacteriome (Figs. 1, 2). Seven additional isolates matched candidate-core OTUs identified by Birt et al.[4] that were not retained in the final core, while four were assigned the same taxonomy as core OTUs but shared < 97% sequence similarity and were therefore not considered core matches.

Eleven of the 24 recovered core OTUs were represented by a single isolate, whereas the remaining 13 were each represented by between two and ten isolates. For each OTU represented by multiple isolates, a single representative was selected based on average nucleotide identity (ANI), genome quality, and similarity of the genome-derived 16S rRNA gene sequence to the corresponding core OTU sequence (Fig. 2; Table 1; Table S4). Hereafter, we refer to these representative strains as core isolates.

Table 1

Taxonomic assignments and structural features of the 24 representative core isolate genomes. Isolates are identified by the corresponding core OTU from which they were recovered. Percentage identity indicates 16S rRNA gene sequence identity between each isolate genome and the corresponding core OTU taxonomy and closest reference genomes were assigned using GTDB-Tk (r214), and genome completeness and contamination were estimated using Che characteristics include genome size, number of contigs, N50, GC content, coding sequences (CDS), and numbers of rRNA, tRNA, and tmRNA genes. An asteri Tk did not assign a closest species because the genome fell outside the predefined ANI radius.

Core OTU	Tissue	16S identity (%)	GTDB-tk closest placement		NCBI TaxID	Genome size (Mb)	Contigs	CheckM2		N50 (bp)	G
			Taxonomy	Reference				Completeness (%)	Contamination (%)		
[2]	Roots	1	*Agrobacterium leguminum*	GCF_015704895.1	2792015	5.16823	32	100	0.52	8	0
[3]	Roots	1	*Priestia zanthoxyli*	GCF_009497655.1	1404	5.151467	183	100	0.66	27	0
[4]	Roots	0.98	*Sphingomonas spp.*	*	13687	4.792145	147	100	0.38	23	0
[5]	Roots	0.99	*Herbaspirillum spp.*	*	963	5.808845	107	100	0.37	9	0
[8]	Leaves	0.98	*Pantoea dispersa*	GCF_014155765.1	59814	4.736547	6	100	0.02	1	0
[9]	Roots	1	*Bradyrhizobium spp.*	*	374	8.214317	582	100	0.92	64	0
[10]	Roots	1	*Ralstonia spp.*	*	48736	5.918151	126	99.92	0.7	18	0
[11]	Roots	1	*Rhizobium hainanense*	GCF_900094555.1	52131	7.316842	324	99.99	2.06	11	0
[12]	Roots	0.99	*Sphingomicrobium spp.*	*	13687	2.455545	1	99.97	0.04	1	0
[16]	Roots	0.98	*Mycobacterium spp.*	*	1763	6.704982	323	100	0.41	47	0
[17]	Leaves	0.98	*Peribacillus frigoritolerans*	GCF_001636405.1	450367	5.425008	411	100	0.23	40	0
[18]	Roots	1	*Variovorax paradoxus_A*	GCF_000382045.1	1157708	7.44426	232	100	1.68	34	0
[20]	Roots	1	*Dyella_B spp.*	*	231454	5.541538	563	99.99	0.28	95	0
[21]	Roots	0.99	*Sphingobium spp.*	*	165695	3.737933	30	99.94	0	7	0
[25]	Roots	0.97	*Streptomyces sp003947455*	GCF_003947455.1	2203206	8.281414	755	100	0.54	110	0
[35]	Roots	1	*Bacillus altitudinis*	GCF_000691145.1	1178544	3.738146	48	100	0.03	8	0
[42]	Leaves	0.98	*Pseudomonas_B sp913774235*	GCA_913774235.1	114707	5.049527	51	100	0	9	0
[61]	Leaves	1	*Curtobacterium luteum*	GCF_014646995.1	33881	3.669671	1	100	0.05	1	0
[92]	Leaves	0.98	*Methylobacterium spp.*	*	407	6.885214	1082	99.83	0.24	163	0
[98]	Roots	0.98	*Sphingomonas_N spp.*	*	13687	5.297764	306	100	0.47	40	0
[99]	Roots	0.99	*Rhizobium spp.*	*	379	5.630878	2333	93.87	3.6	436	0
[104]	Leaves	1	*Methylobacterium radiotolerans*	GCF_000019725.1	426355	6.65954	1617	99.31	1.6	211	0
[131]	Roots	0.99	*Casimicrobium spp.*	*	674703	7.695179	262	100	1.43	45	0
[169]	Roots	0.97	*Sphingomicrobium sp023389105*	GCA_023389105.1	1977087	2.561232	5	100	0.34	1	0

* Genome not assigned by GTDB to closest species as it falls outside its pre-defined ANI radius

In total, this work generated a biobank of 62 isolates corresponding to 24 of the 36 banana core OTUs defined by Birt et al.[4]. These biobanked isolates are available on request.

3.3 The biobank captures a substantial fraction of the dominant banana microbiome

Targeted culturomics successfully recovered representatives of 24 of the 36 banana core OTUs (66.7%). We next assessed the extent to which these cultivated taxa captured the numerically important fraction of the banana microbiome in field-grown plants. We first examined the representation of the 24 cultivated core OTUs among the "key constituents" identified by Birt et al.[4]. These key constituents were defined as OTUs present in ≥ 50% of field-grown plants in at least one compartment at ≥ 0.5% relative abundance.

Across plant compartments, the cultivated set captured a substantial proportion of these key constituents, both numerically (ectorhizosphere, 26%; endorhizosphere, 67%; pseudostem, 40%; leaf, 29%) and in terms of their combined relative abundance (ectorhizosphere, 53%; endorhizosphere, 73%; pseudostem, 46%; leaf, 42%) (Fig. 3). Recovery was particularly high in the endorhizosphere, where the cultivated set encompassed 67% of key constituent taxa representing 73% of their combined relative abundance. Thus, the biobank not only captures two-thirds of the sequence-defined banana core bacteriome, but also encompasses a substantial fraction of the dominant bacterial populations associated with field-grown banana.

Because core taxa differed substantially in abundance, we next asked whether the cultivated set was weighted towards the more abundant members of the banana core microbiome. We ranked the 36 core OTUs by mean relative abundance within each of four plant compartments of field-grown banana: ectorhizosphere, endorhizosphere, pseudostem, and leaf. In each compartment, the most abundant core OTU was represented by a cultivated isolate (OTUs 12, 5, 2, and 4, respectively) (Fig. 4). Overall, the cultivated set captured 60%, 100%, 90%, and 70% of the ten most abundant core OTUs in the ectorhizosphere, endorhizosphere, pseudostem, and leaf, respectively. Recovery was particularly striking in the endorhizosphere, where isolates were obtained for all 14 of the most abundant core OTUs. Although some highly ranked core OTUs, including OTUs 13 and 73, were not recovered, the biobank captured much of the dominant fraction of the banana core microbiome. Together, these analyses highlight both the substantial coverage achieved by the biobank and the core taxa that remain to be recovered.

3.4 Taxonomic and structural annotation of core isolate genomes

The 62 core isolates were assigned to 20 genera spanning the Proteobacteria, Actinobacteriota, and Firmicutes. Together, these represent three of the five phyla present among the 36 core OTUs defined by Birt et al.[4] (Figs. 2, 5). For 13 of the 24 recovered core OTUs, at least one isolate exhibited 100% 16S rRNA gene sequence identity to the corresponding core OTU sequence reported by Birt et al.[4] (Figs. 2, 5).

Genome assemblies were high quality for all but three isolates (16 − 3, 17 − 2, and 17 − 4), which were classified as medium quality but still showed high completeness and low contamination (completeness ≥ 93.96%, contamination ≤ 6.56%) (Fig. 5). Overall, the genome assemblies provided a robust basis for downstream taxonomic and functional analyses.

3.5 Representative core isolates

Pairwise ANI comparisons among the 62 isolate genomes showed that genomes matching the same core OTU were consistently more similar to one another than to genomes matching different core OTUs (Fig. 5). This clustering supported the selection of a single representative isolate from each core OTU for downstream genomic and functional characterisation. Using the criteria described in the Methods, one representative isolate was therefore selected for each of the 24 cultivated core OTUs (Table 1; Fig. 5).

3.6 Structural annotation of core isolate genomes

The representative isolate genomes contained 2,460–7,746 coding sequences, 1–19 rRNA genes, 46–85 tRNA genes, and 1–2 tmRNA genes (Table 1; structural information for non-representative genomes is provided in Table S4). All representative genomes had ≤ 5% contamination and ≥ 90% completeness (Fig. 5), supporting confidence in downstream functional annotation. The only representative genome with < 99% completeness was that matching core OTU99 (93.87% completeness), which may increase the likelihood of false negatives in its functional annotation. The two *Sphingomicrobium* isolates, corresponding to OTUs 12 and 169, had the smallest draft genomes, at approximately 2.5 Mb and ~ 2,500 coding sequences (Table 1), consistent with genome sizes reported for other members of this genus[46].

3.7 Functional annotation of core isolate genomes

Genome sequencing of the 24 representative core isolates enabled assessment of their functional potential for plant-associated processes and substrate utilisation. Collectively, the genomes contained genes and pathways associated with biofertilisation, biostimulation, and the production of hydrolytic enzymes that may facilitate access to a range of plant- and soil-derived substrates (Figs. 6, S1).

Genes associated with nutrient acquisition and transformation were widespread but unevenly distributed across isolates. The complete nitrogen fixation operon (*nifDHK*) was detected in two genomes. The quinoprotein glucose dehydrogenase gene (*gcd*), a marker associated with phosphate solubilisation[16], was identified in five isolates. Seven genomes contained either *tsdA* or *soxAX*, genes involved in thiosulfate oxidation, and two genomes encoded the complete SOX system. Siderophore biosynthetic gene clusters were identified in seven isolates using antiSMASH, while genes encoding the zinc transport system (*znuABC*) or the manganese/zinc/iron transport system (*troBCD*) were detected in ten isolates.

Genes associated with phytohormone modulation were present in all but one representative isolate. The ACC deaminase gene (*acdS*) was identified in nine genomes. Complete pathways for indole-3-acetic acid (IAA) synthesis from tryptophan were annotated in 13 isolates. The tryptophan side-chain pathway was present in all six Gammaproteobacteria isolates, whereas complete indole-3-acetaldoxime pathway 1 and/or 2 modules were identified in Proteobacteria and Actinobacteriota isolates. The *amiE* gene, which catalyses the terminal step of the indole-3-acetamide pathway, was present in all isolates, although no genome contained the complete set of genes for this pathway. Genes required for tRNA-dependent cytokinin biosynthesis were identified in two isolates. Terpene biosynthetic gene clusters were detected in all but one isolate, whereas only a single isolate carried a phenazine biosynthetic gene cluster.

Genes encoding hydrolytic enzymes were also common, indicating broad genomic potential for utilisation of plant- and soil-derived carbon substrates. One genome contained both the beta-glucosidase gene (*bglX*) and an endo-1,3(4)-beta-glucanase gene (*EBG*), while a further 13 contained *EBG* alone. Genes associated with amylase activity, including *AMY*, *treS*, and *cd*, were detected in 18 isolates. DRAM annotation identified carbohydrate-active enzymes (CAZymes) associated with amorphous cellulose degradation in 21 isolates and pectin degradation in 13 isolates. In contrast, none of the representative genomes contained the *chiA* gene associated with chitinase production (Fig. S1).

3.8 Identification of core isolates with in vitro suppressive activity against Foc-R1 and Foc-SR4

To identify representative core isolates with antagonistic activity against Foc, we screened all 24 representatives in dual-culture assays against Foc-R1 and Foc-SR4. Eight isolates (8, 9, 10, 20, 42, 61, 98, and 99) significantly inhibited the growth of both Foc races, while isolate 3 significantly inhibited Foc-SR4 only (Fig. 7). Isolates corresponding to core OTUs 8, 10, 42, and 61 showed the strongest inhibition, reducing Foc-R1 growth by 53%, 50%, 43%, and 52%, respectively, and Foc-SR4 growth by 51%, 50%, 40%, and 31%, respectively (Fig. 7).

3.9 Foc-R1-suppressive isolates reduced rhizome necrosis severity in planta

Having identified representative core isolates with strong in vitro antagonistic activity against Foc, we next assessed whether these effects translated into reduced disease severity in banana plants inoculated with Foc-R1. Rhizome necrosis differed significantly among cross-sections and treatments, whereas the cross-section:treatment interaction was not significant (ANOVA, cross-section: $F = 3.08$, $P = 0.031$; treatment: $F = 4.73$, $P = 0.002$; cross-section:treatment: $F = 0.75$, $P = 0.699$). Across Foc-R1-inoculated plants sampled at day 169, rhizome necrosis decreased from the base of the rhizome towards the pseudostem (Fig. 8), consistent with infection progressing from the roots through the rhizome. Plants inoculated with isolates corresponding to OTUs 8 or 10 had significantly less necrosis than mock-inoculated controls in the first rhizome cross-section and when necrosis was averaged across all four cross-sections (Fig. 8). Necrosis was also lower in the second, third, and fourth cross-sections of plants inoculated with isolates 8 and 10, although these differences were not significant. At harvest, only one plant in each of the isolate 8 and 10 treatments showed detectable necrosis (Fig. 8D). Plants inoculated with isolate 42 also showed lower necrosis than mock-inoculated controls across all four cross-sections and for mean necrosis overall, but these reductions were not significant (Fig. 8). Thus, the strongest evidence of *in planta* suppression of Foc-R1 was observed for representative core isolates 8 and 10. All plants not inoculated with Foc-R1 remained healthy, with no detectable rhizome necrosis (Fig. 8D).

4. Discussion

4.1 Recovering the banana core microbiome as a cultured resource

Sequence-based surveys have transformed our understanding of plant microbiomes, but their ecological and translational value is often limited by the lack of cultured representatives. This is especially true in crops, where the goal is not only to describe host-associated communities, but also to understand their functions and ultimately harness them to improve plant performance[2,3]. Here, we used targeted culturomics to recover and biobank cultured representatives of the banana core bacteriome previously defined by Birt et al.[4]. We recovered isolates corresponding to 24 of the 36 banana core OTUs, representing 66.7% of the core microbiome. Importantly, this substantial taxonomic recovery translated into even greater coverage of the abundant fraction of the banana microbiome. Relative to the microbiomes of field-grown banana plants characterised by Birt et al.[4], the 24 cultivated core OTUs accounted for a substantial proportion of the combined relative abundance of key constituents, ranging from 53–73% below ground and 42–46% above ground. The cultivated set also captured 60%, 100%, 90%, and 70% of the ten most abundant core OTUs in the ectorhizosphere, endorhizosphere, pseudostem, and leaf, respectively, including the most abundant core OTU in every compartment. Together, these results show that the biobank captures both a substantial proportion of the banana core bacteriome and much of its dominant fraction, creating a cultured resource for experimental interrogation and microbiome-based crop improvement.

4.2 High-throughput limiting dilution enables targeted recovery of core taxa

A central result of this study was the effectiveness of high-throughput limiting dilution (HLD) culturomics for recovering targeted members of the banana microbiome. HLD recovered almost all bacterial OTUs detected across the isolation experiments, including most of the core taxa ultimately captured in the biobank. This high recovery likely reflects, at least in part, the scale of the approach: 38,880 HLD cultures could be screened in parallel, greatly increasing the opportunity to recover specific taxa from complex plant-associated communities. Despite the substantially greater diversity recovered by HLD, cultivation method explained only a small proportion of variation in phylum-level community composition, suggesting that the broad taxonomic profiles recovered by HLD and traditional plating were relatively similar. In this respect, our findings align with prior work showing that sequence-informed, highly parallel cultivation can recover substantial fractions of plant microbiomes that would be poorly represented using standard agar plate-based approaches alone[20,21,47]. More broadly, our results demonstrate the value of combining prior ecological knowledge with highly parallel cultivation and sequence-based screening to translate sequence-defined crop microbiomes into culture collections.

4.3 The biobank captures much of the dominant fraction of the core

The value of the biobank lies not only in the proportion of the banana core recovered, but also in the ecological characteristics of the taxa it contains. The cultivated set was strongly represented among the abundant and prevalent bacterial populations associated with field-grown banana, providing access to taxa that repeatedly constitute substantial components of the host-associated microbiome. This does not establish that these 24 core OTUs are the most functionally important members of the banana microbiome, nor does it imply that less abundant or non-core microorganisms do not contribute substantially to host function[48]. Rather, their persistence and abundance make the cultivated core taxa logical priorities for further investigation. This is particularly

relevant because the biobank converts these ecologically important but previously sequence-defined taxa into experimentally tractable resources. Genome sequences can reveal their functional potential, but cultured isolates allow those predictions to be tested directly and enable investigation of the environmental conditions that regulate their growth and activity. This creates opportunities not only to identify strains or synthetic communities for direct inoculation, but also to determine how soil and crop management might favour beneficial functions of core taxa already resident within banana production systems.

4.4 The remaining uncultured core still warrants attention

The 12 core OTUs that remain without cultured representatives are important targets for future cultivation efforts. Several were among the more abundant core members within particular plant compartments and may therefore make important ecological contributions to the banana microbiome. Their failure to be recovered may reflect a combination of factors, including slow growth, low abundance following tissue processing, sensitivity to the cultivation conditions used, or dependence on nutritional or biotic interactions not reproduced in our assays. Future efforts to isolate these taxa could therefore benefit from longer incubation periods, media that better mimic plant-associated nutrient environments, or more targeted enrichment approaches[49–52]. For example, selective enrichment strategies may improve recovery of the *Nitrospira*-affiliated core OTU 28[53–55], whereas extended incubation may be more suitable for slow-growing taxa such as the *Gaiella*- and Gemmatimonadetes-affiliated core OTUs[56–60]. For poorly characterised core taxa, bespoke cultivation conditions informed by metagenome-resolved functional predictions may be especially useful[61,62]. In future, such approaches could be coupled with targeted cell sorting or hybridisation-based screening to further increase recovery of the remaining uncultured core members[63–65].

4.5 Genome annotation reveals broad plant-beneficial potential

The biobank generated here provides an experimental resource for testing the long-standing hypothesis that core taxa contribute disproportionately to host function[66]. Genome annotation revealed that the representative isolates collectively possess broad genetic potential for biofertilisation, biostimulation, and substrate utilisation. These included genes associated with nitrogen fixation, phosphate solubilisation, sulphur oxidation, siderophore production, zinc transport, ACC deaminase, indole-3-acetic acid biosynthesis, cytokinin-associated pathways, terpene biosynthesis, and multiple hydrolytic enzymes. Taken together, these annotations identify a broad range of functions that could contribute to plant–microbe interactions. Importantly, genomic potential does not demonstrate that these functions are expressed or ecologically relevant under field conditions. The value of the biobank is that these predictions can now be tested experimentally, allowing genomic hypotheses to be linked directly to isolate phenotype.

Several specific patterns are noteworthy. Two representative isolates, corresponding to core OTUs 5 (*Herbaspirillum*) and 9 (*Bradyrhizobium*), encoded the nitrogenase structural genes *nifDHK*, highlighting a plausible route by which members of the banana core microbiome could contribute to host nitrogen nutrition. This is potentially relevant because nitrogen availability is a major constraint on banana production[67]. Core OTU 5 may be especially interesting in this respect because it is core to the endorhizosphere, where close association with plant tissues and comparatively low oxygen conditions may favour nitrogen fixation[68,69]. Multiple isolates also encoded traits linked to phosphate, sulphur, iron, and zinc acquisition, suggesting that future work could explore whether the abundance or activity of particular rhizosphere-associated core taxa can be enhanced to improve nutrient-use efficiency in banana systems[70,71]. More broadly, the presence of ACC deaminase, IAA-associated pathways, cytokinin-related genes, terpene biosynthetic gene clusters, and phenazine biosynthesis in subsets of the cultivated core supports the hypothesis that banana-associated core taxa may influence plant growth and stress responses through multiple mechanisms[1,72–76]. Importantly, failure to detect these specific genes or pathways does not necessarily indicate an absence of the corresponding function. Core isolates may possess alternative or currently unrecognised genetic mechanisms underlying these traits. For example, cytokinin biosynthesis genes were not annotated in the *Methylobacterium*, *Bradyrhizobium*, *Bacillus*, or *Rhizobium* core isolates, despite cytokinin production having been reported among species from these genera[72].

4.6 Core isolates include candidates for suppression of Fusarium wilt

Because empirical validation of every predicted trait was beyond the scope of this study, we focused on one agriculturally important function: suppression of *Fusarium oxysporum* f. sp. *cubense* (*Foc*). This was a logical target because Fusarium wilt remains one of the most significant threats to banana production globally, and disease suppression is a complex phenotype that cannot be reliably predicted from genome annotation alone. In vitro assays identified several representative core isolates with antagonistic activity against both *Foc*-R1 and *Foc*-SR4, with isolates 8, 10, 42, and 61 showing the strongest inhibition. When tested in planta, isolates 8 and 10 significantly reduced *Foc*-R1-associated rhizome necrosis, while isolate 42 showed a non-significant trend towards suppression. These findings provide proof-of-concept that targeted cultivation of the banana core microbiome can recover taxa with disease-suppressive activity and demonstrate how the biobank can be used to move from sequence-defined ecological targets to experimentally validated functions with potential translational relevance for crop protection.

The contrast between in vitro and in planta performance also provides an important ecological lesson. Despite strong inhibition of Foc in dual culture, isolate 61 did not significantly reduce disease symptoms in plants. One plausible explanation is ecological mismatch. The corresponding core OTU is associated primarily with the phyllosphere and occurs at low abundance in other compartments[4], suggesting that it may be poorly adapted to survival or competition in the rhizosphere environment where *Foc* infection is initiated. By contrast, core OTU 42 is associated with the rhizome and occurs at low abundance in the ectorhizosphere, placing it in closer ecological proximity to the tissues affected during *Foc* infection. Although establishment of these isolates was not measured here, their contrasting compartment associations provide a testable hypothesis for the differences observed between *in vitro* and *in planta* performance. More broadly, these results emphasise that pathogen suppression measured *in vitro* is not, on its own, an adequate basis for selecting microbial inoculants. Successful candidates must also establish and function under the ecological conditions in which disease develops[18]. One advantage of working from a core microbiome framework is that it provides ecological context for interpreting these outcomes and for prioritising taxa that are both functionally interesting and appropriately positioned within the host.

4.7 The biobank provides a foundation for microbiome engineering in banana

The value of the biobank extends well beyond single-isolate screening. By converting much of the dominant banana core microbiome into a cultured resource, it is now possible to address mechanistic questions that were previously difficult to investigate experimentally. These include how persistent banana-associated taxa interact with one another, whether core members exhibit functional redundancy or complementarity, how they respond to host genotype and soil conditions, and whether their abundance or activity can be manipulated through management rather than direct inoculation alone. Genome-guided predictions combined with isolate-based experiments can identify environmental conditions that favour particular core taxa and test colonisation, competition, metabolic division of labour, and emergent traits in synthetic communities. Such questions are particularly relevant for microbiome engineering, where durable outcomes may depend less on individual strains than on ecologically compatible consortia and management strategies that reinforce resident beneficial taxa[18,77].

Conclusions

This study shows that a large and ecologically important fraction of the banana core microbiome can be recovered through targeted culturomics and converted into a biobank of genomically characterised isolates. The resulting resource captures many of the most abundant core taxa across plant compartments, enables experimental testing of their functional roles, and has already yielded proof-of-concept candidates for suppression of Fusarium wilt. By bridging sequence-based core microbiome ecology and experimental microbiology, this work provides a foundation for mechanistic studies and microbiome-based strategies to improve banana health and productivity.

Declarations

Conflict of Interest

The authors declare no conflicts of interest.

Funding

This research was supported by the Australian Centre for International Agricultural Research through the research project HORT/2018/192.

Author Contribution

PGD and OMP designed the study. HRLG collected plant samples. OMP, RL, and HRLG performed experiments. OMP, JS, and PGD analysed data. PGD and ABP secured funding. OMP and PGD wrote the paper with input from all authors.

Acknowledgement

We thank Wayne O'Neill (Queensland Department of Primary Industries) for providing the Fusarium isolates used in this study.

Data Availability

The sequence data associated with this study is available from the European Nucleotide Archive (ENA) under Project accession PRJEB115327. Prokka genome annotation associated with this study is available from Zenodo under 10.5281/zenodo.21281317. Supplementary information supporting the study is provided with the article, and raw antiSMASH, DRAM and KofamScan annotation outputs for the representative core isolate genomes are provided as Supplementary Data.

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Figures

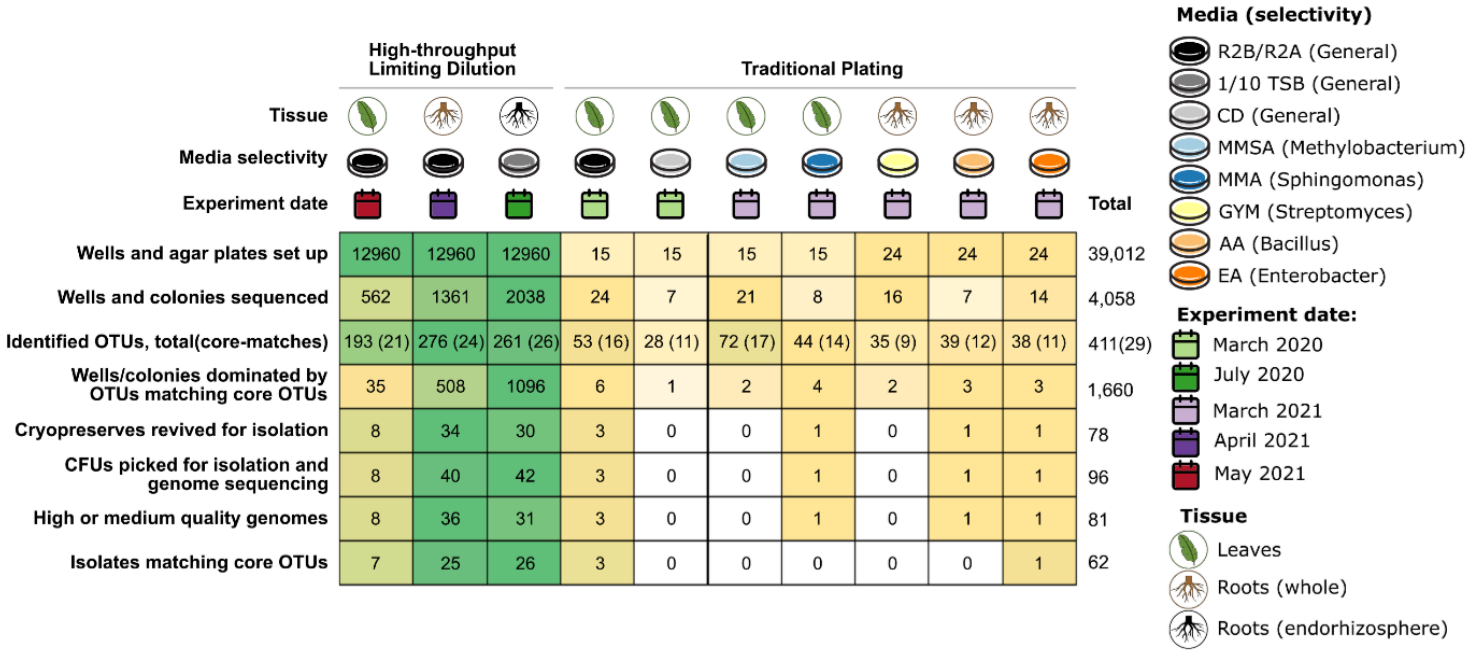


Figure 1

Conditions and outcomes of five bacterial isolation experiments used to recover members of the banana core microbiome. Isolation experiments were conducted on five dates, with tissue type, cultivation medium and isolation method shown for each cultivation condition. Numbers show progression through the isolation workflow, from culture vessels established and screened by 16S rRNA gene metabarcoding through identification of core-matching OTUs, cryopreservation, colony purification, genome sequencing, genome quality assessment, and verification of isolates corresponding to banana core OTUs. Values in parentheses indicate the number of OTUs matching banana core OTUs. Totals across all five experiments are shown to the right.

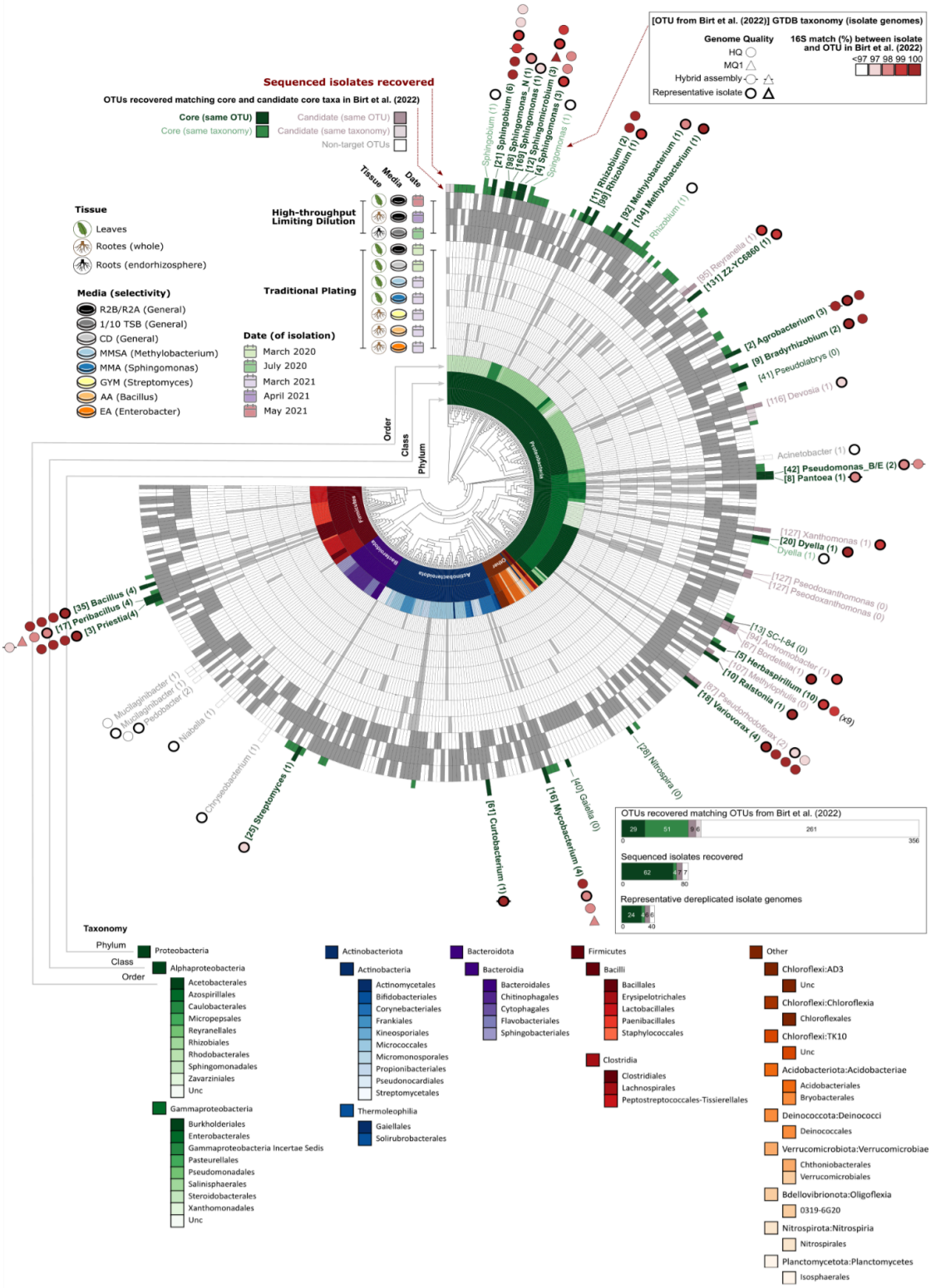


Figure 2

Taxonomic diversity of cultured bacterial OTUs and recovery of isolates corresponding to the banana core microbiome. The central phylogeny represents bacterial OTUs identified by 16S rRNA gene sequencing across the five isolation experiments, with the three innermost coloured rings indicating phylum, class, and order assignments (SILVA v138 SSU27). The ten outer tracks indicate detection (grey) or absence (white) of each OTU under the cultivation conditions shown, comprising different combinations of isolation method, tissue, medium, and experimental date. Peripheral cells identify recovered OTUs corresponding to core or candidate-core taxa defined by Birt et al.[4]. Dark green indicates ≥97% 16S rRNA gene sequence similarity to a core OTU and light green indicates shared taxonomy but <97% sequence similarity; equivalent matches to candidate-core OTUs are shown in dark and light grey, respectively. The outermost cells indicate OTUs for which pure isolates were recovered. For sequenced isolates, annotations around the perimeter show the corresponding core or candidate-core OTU ID, GTDB taxonomy (r214[32]), and number of isolates recovered. Symbols indicate genome quality and 16S rRNA

gene sequence similarity to the corresponding OTU from Birt et al.[4], with bold outlines identifying the representative isolate selected for each cultivated core OTU. The inset summarises recovery of core, candidate-core, and other taxa across cultured OTUs, sequenced isolates, and representative genomes.

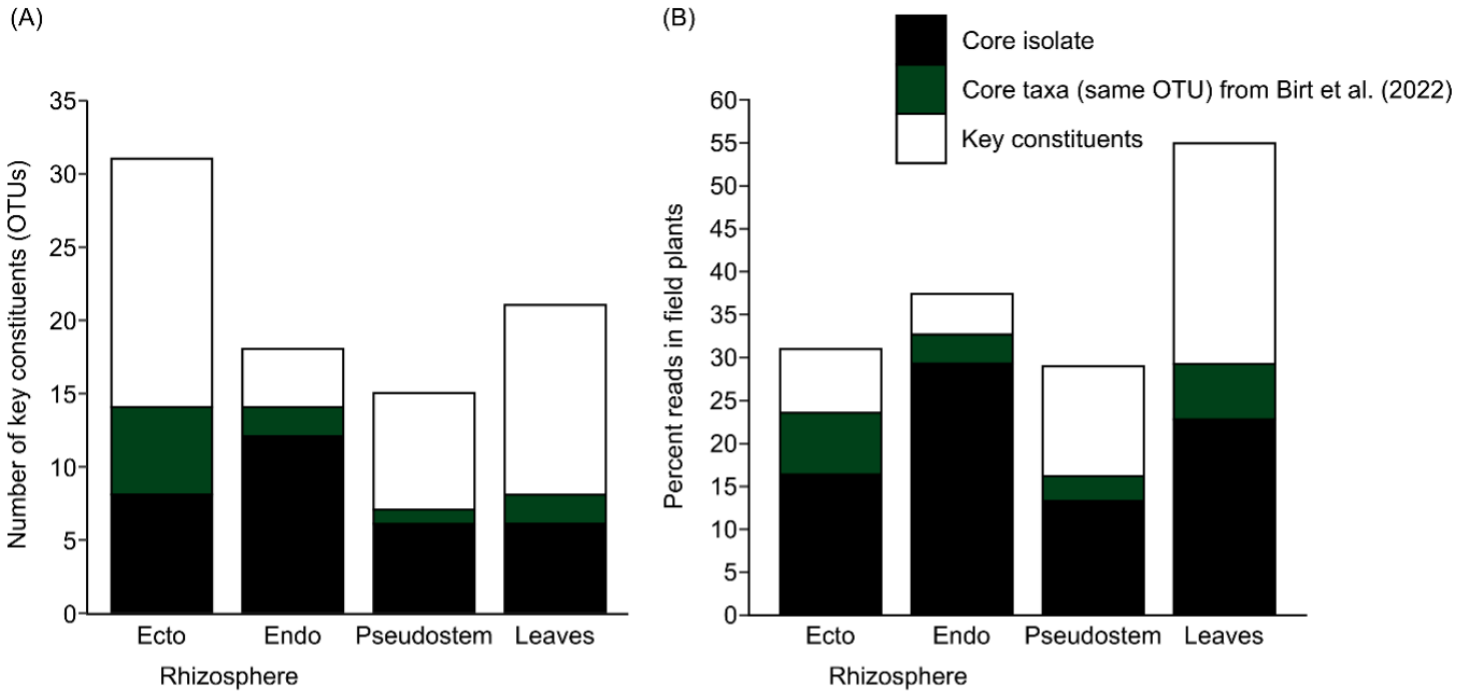


Figure 3

Representation of cultivated core OTUs among key constituents of the banana microbiome in field-grown plants. Key constituents were defined by Birt et al.[4] as OTUs present in ≥50% of field-grown plants at ≥0.5% relative abundance. (A) Number of key constituent OTUs within each plant compartment, showing those corresponding to banana core OTUs (green) and the subset for which isolates were recovered in this study (black). (B) Combined relative abundance of key constituent OTUs in field-grown plants, showing the contribution of core OTUs (green) and the cultivated subset (black). White portions of the bars represent key constituents that were not members of the banana core microbiome.

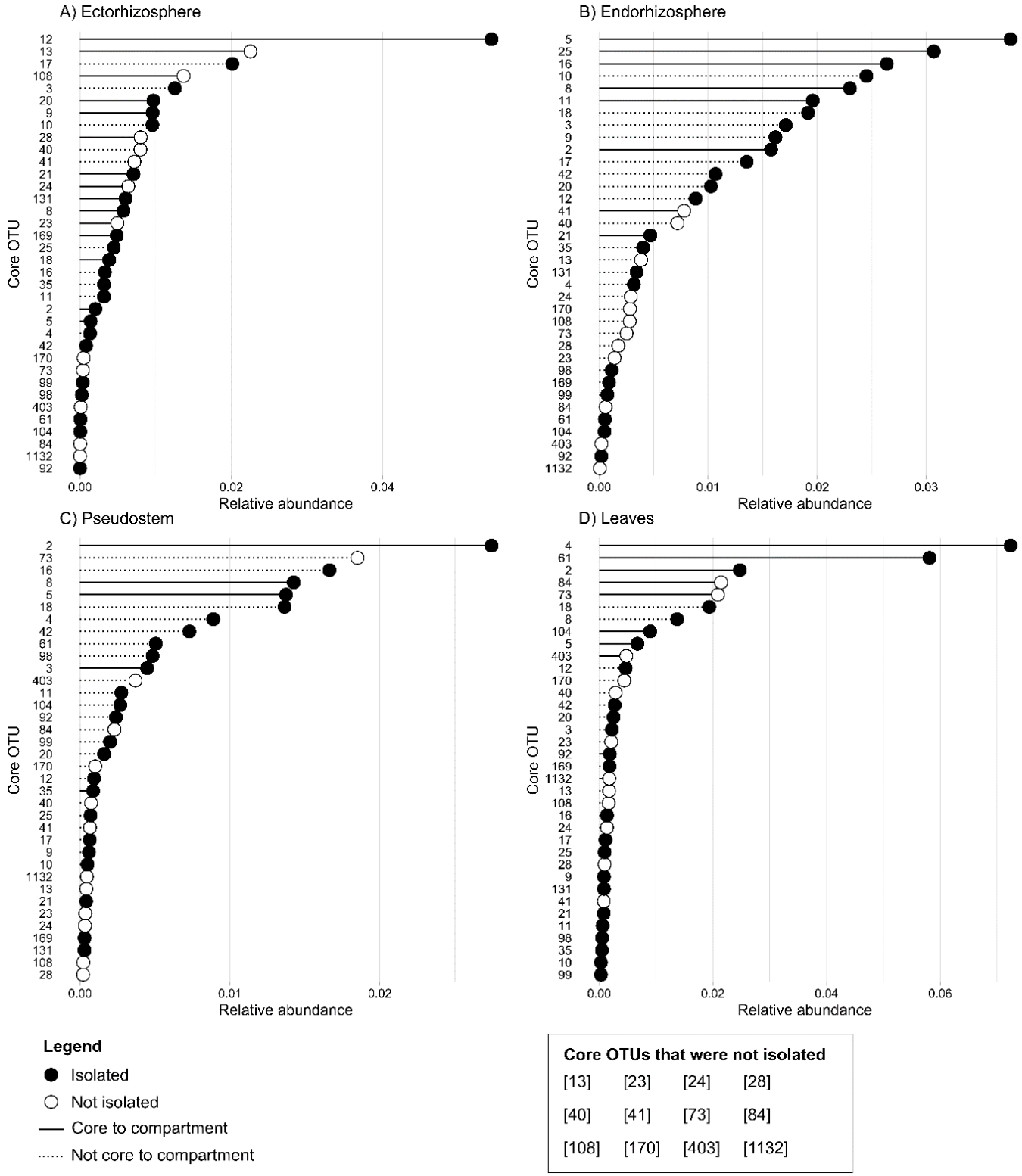


Figure 4

Relative abundance and cultivation status of the 36 banana core OTUs across plant compartments. Mean relative abundance of each core OTU in the (A) ectorhizosphere, (B) endorhizosphere, (C) pseudostem, and (D) leaves of field-grown *Musa* spp., based on data from Birt et al.[4]. OTUs are ranked from highest to lowest mean relative abundance within each compartment. Filled circles indicate core OTUs for which isolates were recovered in this study, whereas open circles indicate core OTUs that remain uncultured. Solid lines indicate OTUs classified as core within the corresponding plant compartment by Birt et al.4, whereas dotted lines indicate core OTUs that did not meet the core criterion within that compartment. The 12 core OTUs for which no cultured representative was recovered are listed in the inset.

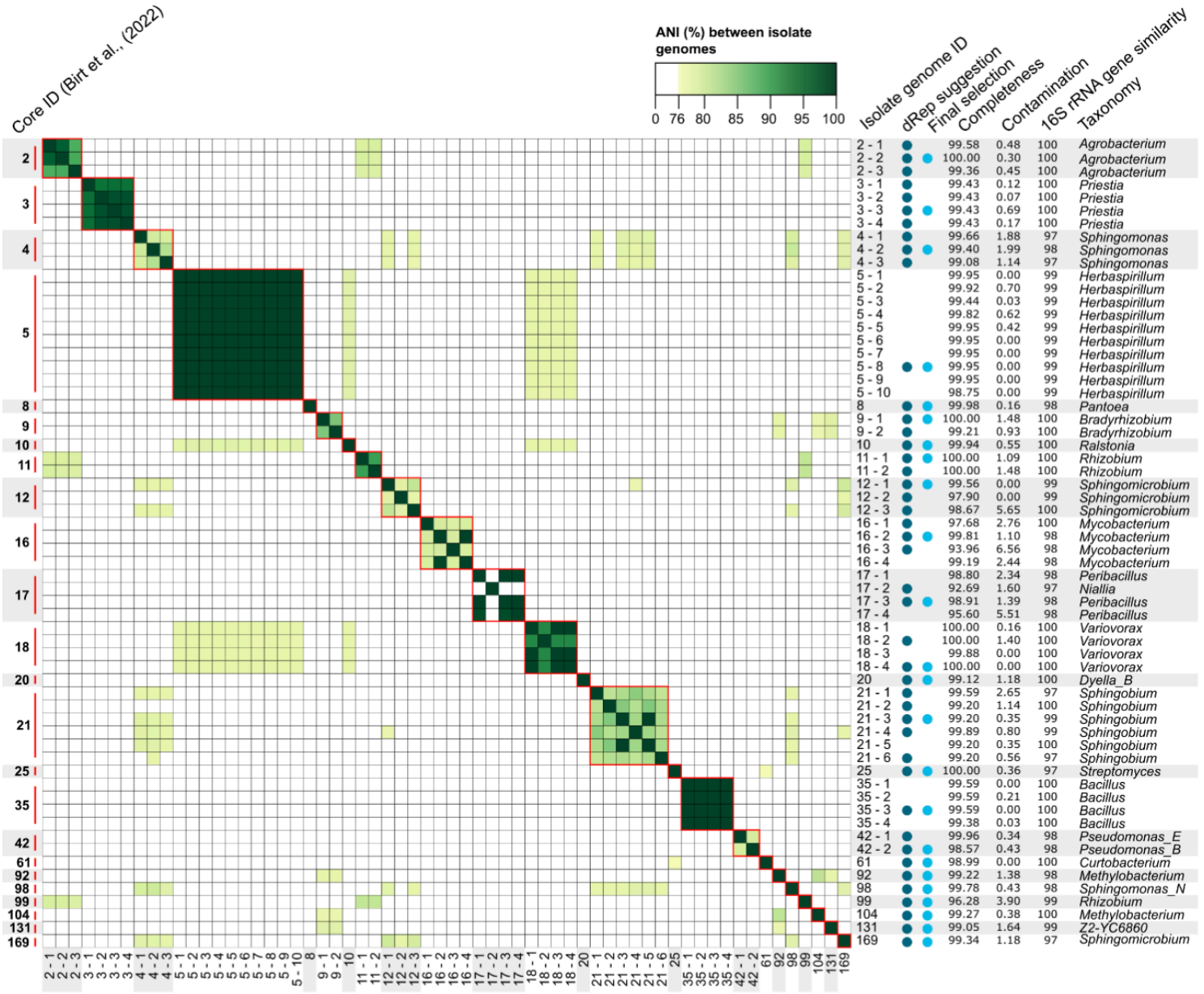


Figure 5

Genomic similarity, quality, and selection of representative isolates corresponding to banana core OTUs. Heatmap shows pairwise average nucleotide identity (ANI) among the 62 isolate genomes matching the 24 banana core OTUs recovered in this study. Isolates are grouped according to their corresponding core OTU from Birt et al.[4], with red boxes delineating isolates assigned to the same core OTU. Where multiple isolates matched the same core OTU, unique isolate identifiers were appended to the core OTU ID. Genome completeness and contamination were estimated using CheckM, and 16S rRNA gene similarity indicates sequence identity between each isolate genome and its corresponding core OTU from Birt et al.[4]. Genome taxonomy was assigned using GTDB (r214). Blue symbols indicate isolates suggested by dRep during genome dereplication and those ultimately selected as the representative isolate for each of the 24 cultivated core OTUs.

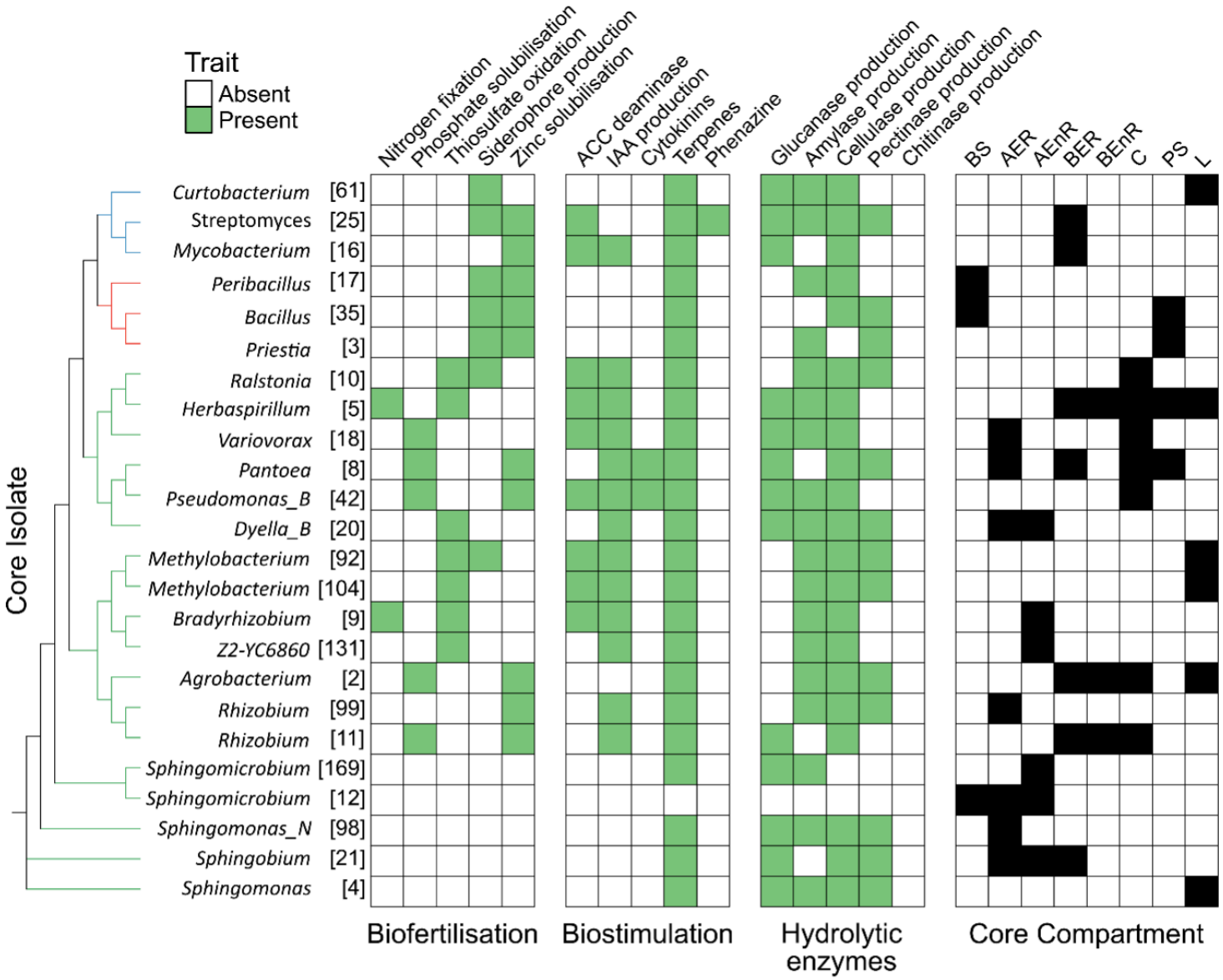


Figure 6

Genomic potential for plant-associated functions among representative banana core isolates. Representative isolates corresponding to the 24 cultivated core OTUs are arranged according to a phylogeny generated using the de novo workflow in GTDB-Tk (r214)[32], with branch colours indicating phylum. Numbers in brackets indicate the corresponding core OTU IDs from Birt et al.[4]. Heatmaps show the presence (green) or absence (white) of genes or pathways associated with biofertilisation, biostimulation, and hydrolytic enzyme activity. The right-hand heatmap indicates the plant compartments in which each corresponding OTU was classified as core by Birt et al.[4] (black, core; white, not core). Abbreviations: BS, belowground soil; AER, aboveground ectorhizosphere; AEnR, aboveground endorhizosphere; BER, belowground ectorhizosphere; BEnR, belowground endorhizosphere; C, corm; PS, pseudostem; L, leaf.

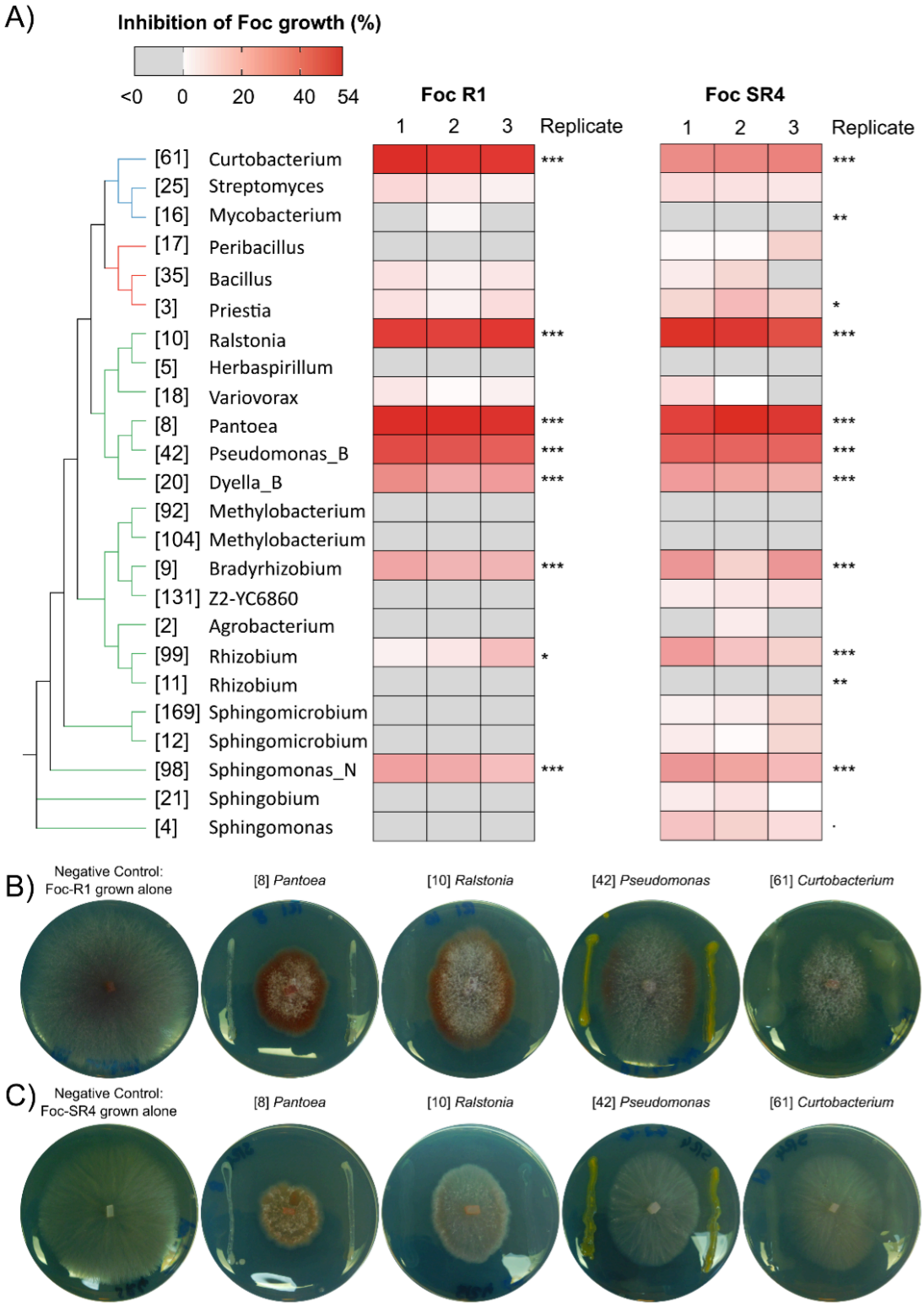
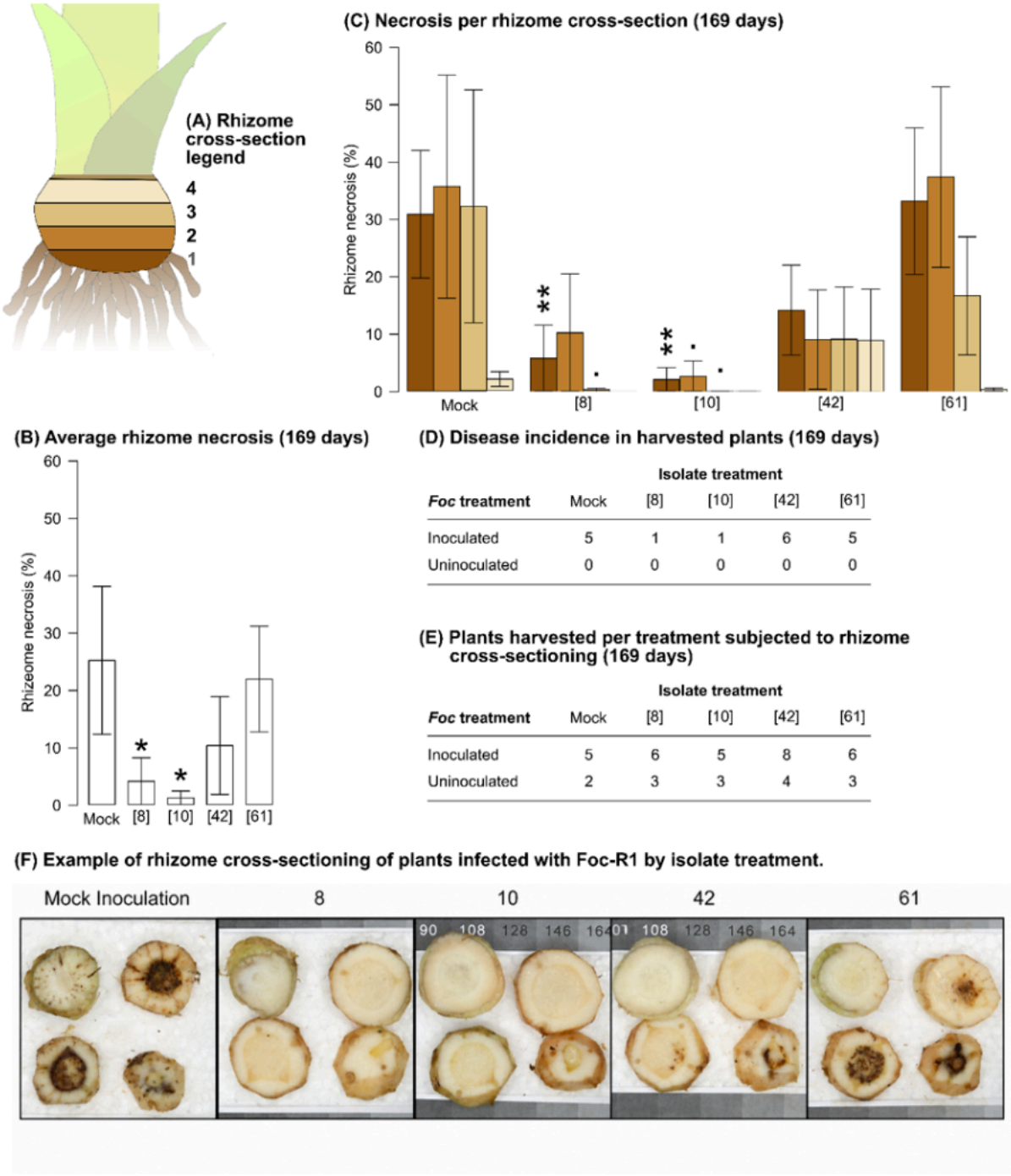


Figure 7

In vitro inhibition of *Foc*-R1 and *Foc*-SR4 by representative banana core isolates. (A) Percentage inhibition of Foc growth in dual-culture assays for each of the 24 representative core isolates (n = 3). Isolates are arranged according to a phylogeny generated using the de novo workflow in GTDB-Tk (r214)[32], with branch colours indicating phylum; numbers in brackets indicate corresponding core OTU IDs from Birt et al.[4]. Asterisks indicate significant differences in *Foc* growth relative to *Foc*-only controls (ANOVA: $P < 0.001$***, $P < 0.01$**, $P < 0.05$*, $P < 0.1$·). (B, C) Representative dual-culture assays showing *Foc*-R1 (B) and *Foc*-SR4 (C) grown alone or with representative isolates corresponding to core OTUs 8 (*Pantoea*), 10 (*Ralstonia*), 42 (*Pseudomonas*), and 61 (*Curtobacterium*).



Isolate treatment

Foc treatment	Mock	[8]	[10]	[42]	[61]
Inoculated	5	1	1	6	5
Uninoculated	0	0	0	0	0

Isolate treatment

Foc treatment	Mock	[8]	[10]	[42]	[61]
Inoculated	5	6	5	8	6
Uninoculated	2	3	3	4	3

Figure 8

Effects of representative banana core isolates on *Foc*-R1-associated rhizome necrosis. (A) Positions of the four rhizome cross-sections used to assess internal necrosis. (B) Mean rhizome necrosis across all four cross-sections in *Foc*-R1-inoculated plants at 169 days after initial isolate inoculation. (C) Rhizome necrosis within each of the four cross-sections at 169 days. Plants were mock-inoculated with water or inoculated with representative core isolates 8, 10, 42, or 61 at days 0 and 20, and *Foc*-R1 was applied at days 54 and 72. Colours correspond to the cross-section positions shown in (A). Symbols indicate differences from the mock treatment ($P < 0.01$**, $P < 0.05$*, $P < 0.1$·) based on the statistical analyses described in the Methods. (D) Number of harvested plants exhibiting rhizome necrosis according to isolate and *Foc*-R1 treatment. (E) Number of plants harvested and assessed by rhizome cross-sectioning in each treatment at 169 days. (F) Representative rhizome cross-sections from *Foc*-R1-inoculated plants receiving each isolate treatment or the mock treatment.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Patersonetal.2026.Supplementaryinformation.docx
- Patersonetal.2026.Additionalcoregenomeinformation.zip