Main Manuscript for

The Natural Products Discovery Center: Release of the First 8490 Sequenced Strains for Exploring Actinobacteria Biosynthetic Diversity

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Abstract

Actinobacteria, the bacterial phylum most renowned for natural product discovery, has been established as a valuable source for drug discovery and biotechnology but is underrepresented within accessible genome and strain collections. Herein, we introduce the Natural Products Discovery Center (NPDC), featuring 122,449 strains assembled over seven decades, the genomes of the first 8490 NPDC strains (7142 Actinobacteria), and the online NPDC Portal making both strains and genomes publicly available. A comparative survey of RefSeq and NPDC Actinobacteria highlights the taxonomic and biosynthetic diversity within the NPDC collection, including three new genera, hundreds of new species, and ~7000 non-RefSeq gene cluster families. Selected examples demonstrate how the NPDC Portal's strain metadata, genomes, and biosynthetic gene clusters can be leveraged using genome mining approaches. Our findings underscore the ongoing significance of Actinobacteria in natural product discovery, and the NPDC serves as an unparalleled resource for both Actinobacteria strains and genomes.

Main Text

Introduction

Natural products (NPs) have played a pivotal role in revolutionizing modern and traditional medicine, serving as the foundation for nearly half of all approved drugs available to combat infectious diseases, cancer, endocrine disorders, and various other ailments. [1] Throughout history, humanity has naively harnessed diverse sources of medicinal NPs, but it was not until the mid-20th century that the pursuit of these invaluable compounds became a targeted effort, spurred by the groundbreaking discovery of streptomycin from the soil-dwelling Actinobacterium *Streptomyces griseus* in 1942. [2, 3]. During this era of rapid discovery, many pharmaceutical companies leveraged their resources to search the globe for the next blockbuster drugs and their associated microbial producers. [4] These efforts resulted in the accumulation of vast bacterial collections, which served as the global basis for drug discovery for decades. Despite the immense success of this approach, a confluence of factors, including high rediscovery rates leading to concerns about remaining NP diversity, advancements in combinatorial chemistry and high-throughput screening, and economic considerations, prompted a shift away from industrial NP exploration and microbial collections. [3, 5] However, with the advent of the genomics era, it became increasingly evident that bacteria, especially prolific taxa such as Actinobacteria, possessed far more biosynthetic diversity than had been predicted prior to genome sequencing. [6]

Actinobacteria, recently renamed as Actinomycetota, [7] are found ubiquitously, spanning environments from soil and plants (e.g., *Streptomyces* and *Micromonospora*) to humans (e.g., *Corynebacterium* and

Mycobacterium). [8, 9, 10] The expansive secondary metabolomes of most non-pathogenic Actinobacteria enable them to rapidly adapt to changes in their unique ecological niches due to highly diverse scaffolds and fine-tuned regulation. [11] Consequently, Actinobacteria have emerged as the most extensively studied bacterial phylum for NP discovery, accounting for more than 60% of all characterized bacterial NPs (Fig. 1A), with its most heterogeneous genus, *Streptomyces*, particularly standing out. [12] Genomic analyses have consistently indicated rich biosynthetic gene cluster (BGC) diversity in many Actinobacteria genera that matches their central role in NP exploration, [6] and yet these bacteria remain disproportionately underrepresented in accessible genome databases such as NCBI's RefSeq (Fig. 1B). [13, 14] Furthermore, most public Actinobacteria genomes belong to a few health- or disease-associated species such as the causative agent of tuberculosis, *Mycobacterium tuberculosis* (Fig. 1B), resulting in the available Actinobacteria genomes harboring only a small fraction of the potential BGC and NP diversity of this phylum. [14] A recent study that published 824 genomes of diverse Actinobacteria has predicted as few as 30-50% of the projected Actinobacteria phylogenetic diversity is represented in public genomic datasets, indicating that much work is remaining for the community. [15]

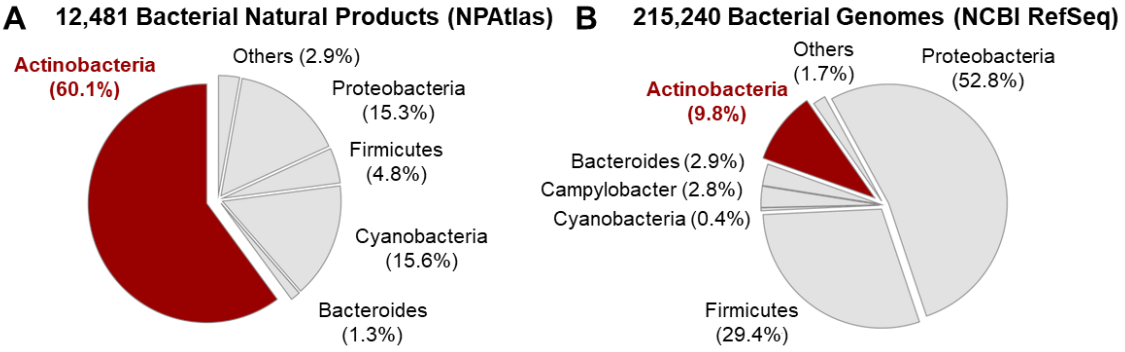

Figure 1. Actinobacteria are overrepresented among bacterial NPs but underrepresented among bacterial genomes. (A) NPs derived from Actinobacteria (red) in comparison to the NPs isolated from other bacterial sources according to NPAtlas. [13] Actinobacteria NPs are primarily isolated from *Streptomyces* (73%), *Micromonospora* (4%), and *Actinomadura* (2%). (B) Actinobacteria genome sequences (red) in comparison to other bacterial genome sequences available in NCBI RefSeq. [16] Actinobacteria RefSeq genomes come primarily from pathogens or other health-associated bacteria such as *Mycobacterium* (45%), *Bifidobacterium* (7%), or *Corynebacterium* (6%). *Streptomyces* make up only a small fraction of Actinobacteria RefSeq genomes (9%).

The available Actinobacteria genomes highlight their immense potential for NP discovery, but translation of their genomic potential into isolated NPs remains a resource- and time-intensive endeavor. [6] However, the scale and speed of genome sequencing can help streamline this process by enabling the rapid categorization of BGCs. By identifying groups of related BGCs, known as gene cluster families (GCFs), redundant efforts can be avoided and the most promising strains among thousands can be strategically prioritized for fermentation. [17, 18] To democratize the NP discovery process, these strains and their genomes must be made readily available, which serves as the inspiration behind the Natural Products Discovery Center (NPDC) at the Herbert Wertheim UF Scripps Institute for Biomedical Innovation & Technology. Comprised primarily of strains collected by industry between the 1940s and the 2010s, the NPDC collection encompasses a total of 122,449 strains, with more than half morphologically assigned as Actinobacteria. [5] Notably, these Actinobacteria outnumber the collective total Actinobacteria deposited in the world's largest public strain collections, including ATCC (3564), NBRC (3266), NRRL (1863), and DSMZ (7223), by several fold (Supplementary Fig. 1). [19, 20, 21, 22]

To develop this unique collection into a community resource for NP discovery and other user-driven needs such as new biocatalytic, biotechnological, or microbiological applications, the NPDC strains are being continuously grown and genome sequenced. Herein, we present a comprehensive analysis of the taxonomic diversity and biosynthetic potential of the initial 8490 sequenced strains. Moreover, we introduce

an online portal that facilitates the exploration of their genomes, particularly their biosynthetic potential for NP production, and access to the physical strains themselves.

Results

The NPDC collection expands the known taxonomic diversity of Actinobacteria.

Genome sequencing facilitates accurate taxonomic classification of bacterial strains and unveiling of evolutionary relationships between microorganisms. Therefore, a subset of the NPDC collection, 8490 bacteria making up 6.9% of the whole collection and expected to be representative of the ecologic, chronologic, and geographic diversity of the NPDC collection, was subjected to short-read genome sequencing (Supplementary Fig. 2-4 and Supplementary Table 1). [5] All of the strains referenced in the manuscript are part of the UF-Scripps Natural Products Discovery Center (NPDC) bacterial strain collection, and all of these bacterial strains fall within the scope of the Nagoya Protocol, which entered into force on October 12, 2014. [23] The median genome in the dataset has a size of 8.1 Mb with 76 contigs and an N50 of 245 kb, making their quality compare favorably to most RefSeq genomes (Supplementary Fig. 5-7). [16] The genome-based taxonomy of the 8940 bacteria was determined by utilizing GTDB-Tk, revealing 7142 (84.1%) as Actinobacteria and the remainder as primarily Firmicutes and Proteobacteria (Fig. 2A and Supplementary Table 2). [24] Although unclear if the non-Actinobacteria strains were the original isolation targets or potential contaminations introduced during various stages of sample collection or handling, this ratio can be extrapolated to indicate the presence of more than 102,000 Actinobacteria within the NPDC collection. Notably, of the original 7055 Actinobacteria phenotypic assignments, 6247 (88.5%) were correctly assigned as Actinobacteria; however, this historical accuracy varies greatly when examined at the genus level (Extended Data Fig. 1 and Supplementary Table 3). While historical classifications for well-studied genera like *Streptomyces* (91.9%) or *Micromonospora* (78.0%) were comparatively accurate, genomic assignments for rarer genera such as *Nocardia* (18.6%), *Streptosporangium* (20.0%), *Actinoplanes* (29.1%), and *Actinomadura* (0.0%) display notable inconsistencies with their historical designations (Extended Data Fig. 1 and Supplementary Table 3).

Upon the public launch of the NPDC in late 2022, the 7142 NPDC Actinobacteria genomes expanded the total RefSeq Actinobacteria genomes by more than 33% and demonstrated the expansive taxonomic diversity encompassed within. These genomes are distributed among 95 Actinobacteria genera (compared to 104 non-Actinobacteria NPDC genera), six of which comprise more than 100 NPDC genomes each (Fig. 2B and Supplementary Fig. 8). The polyphyletic genus *Streptomyces*, considered the largest bacterial genus by species count, has the largest number of NPDC genomes (5386), greatly surpassing the number of RefSeq *Streptomyces* (1990) and underscoring the NPDC's focus on NP-producing genera (Fig. 2B). [25] Of these 95 genera, only 19 have 20 or more characterized NPs in the NPAtlas database. [13] Additionally, ten smaller and rarer genera including *Micromonospora*, *Kitasatospora*, *Nonomuraea*, and *Streptosporangium* minimally doubled their respective numbers of RefSeq genomes (Fig. 2B and Supplementary Fig. 8). All other genera highlighted in Fig. 2 were expanded by at least 40%. The release of these genomes has also bolstered species coverage across Actinobacteria, with the number of newly sequenced species at least doubling the number of RefSeq species in ten genera, including *Streptomyces*, *Kitasatospora*, *Streptosporangium*, and *Kribbella* (Fig. 2B and Supplementary Fig. 8). In total, more than 65% of all 2152 Actinobacteria species present in the NPDC collection were not previously present in the

RefSeq dataset, though RefSeq species are more populated (6.2 strains/species) than new species (1.8 strains/species) among NDPC strains (Supplementary Fig. 9).

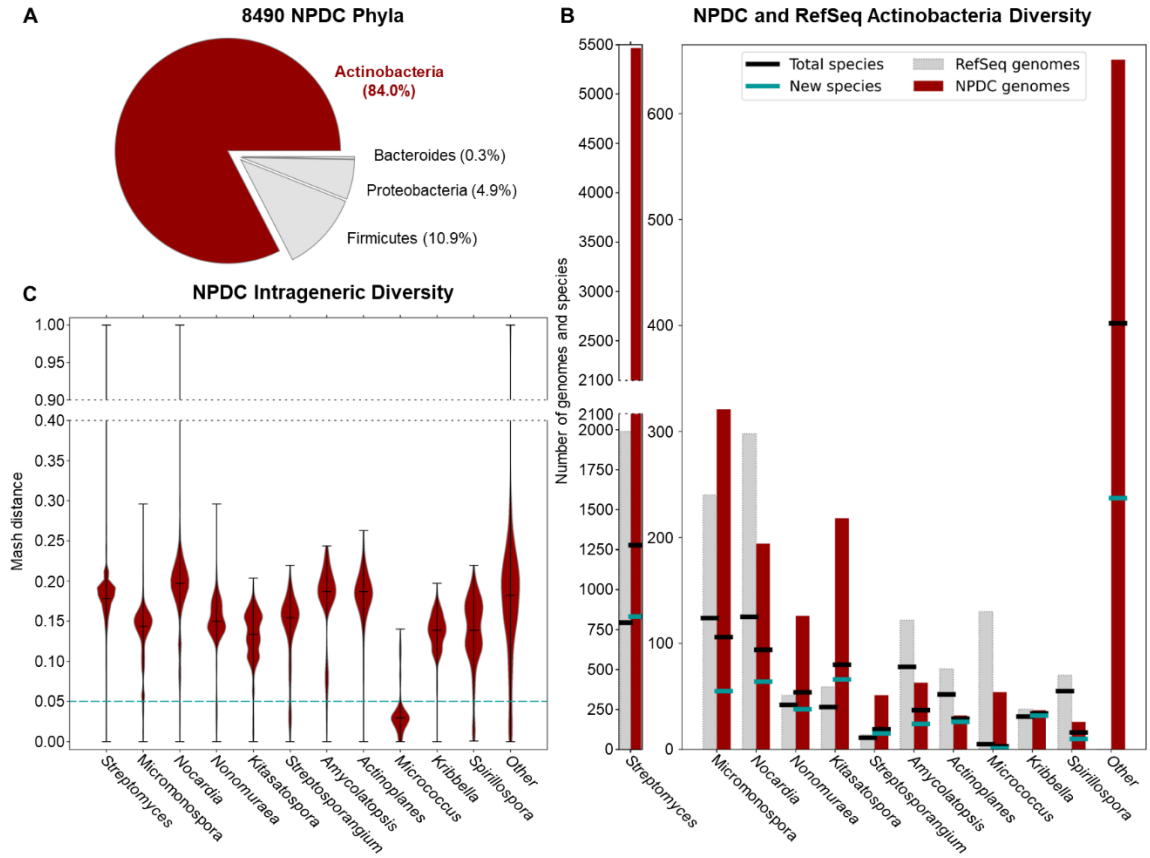

Figure 2. Taxonomic diversity of 8490 genomes from the NPDC. (A) Breakdown of NPDC genomes by phylum, confirming that most NPDC strains are Actinobacteria. (B) Actinobacteria genomes from representative genera in the NPDC (red bars) are compared to genomes from RefSeq (grey bars). Total numbers of species in a genus, based on a 0.05 Mash distance cut-off, are indicated by black lines. Numbers of new species in the NPDC lacking a closely related representative in GTDB are indicated by teal lines. The genomes from non-highlighted genera are combined into a single 'Other' category here, and the breakdowns by individual genera are depicted in Supplementary Fig. 8. (C) Intrageneric diversity in representative, well-populated NPDC genera are displayed by Mash distance distribution. Mash distances smaller than 0.05 are treated as the same species.

The NPDC taxonomic novelty is primarily found in groups of related clades spread throughout the Actinobacteria families best known for NP production (Fig. 3). The observed strain distribution suggests that the NPDC not only contains new species and subspecies but also addresses these preexisting phylogenetic gaps with multiple representatives of related strains. This distribution pattern is particularly evident in *Streptomyces*, wherein most clades are enriched by new NPDC species except for a single clade already covered deeply by RefSeq genomes (Fig. 3). However, some genera, especially those related to health and pathogenicity such as *Mycobacterium*, *Bifidobacterium*, *Corynebacterium*, and *Mycobacteroides*, are notably underrepresented in the NPDC genomes.

For most Actinobacteria genera, the NPDC genomes display high intrageneric diversity, as evidenced by their first quartile Mash distance exceeding 0.05, indicative of distinct species (Supplementary Fig. 10). [26] The Mash distance correlates strongly with average nucleotide identity (ANI), especially in the ANI range of 90-100% (0.1 to 0 Mash distance). [26] Therefore, the high Mash distances observed for most NPDC

Actinobacteria mirror their species-level diversity, especially in genera such as *Nocardia* and *Amycolatopsis* (Fig. 2C). Notably, a few rare genera such as *Micrococcus* are exceptions among Actinobacteria, with most Mash distances below this cutoff, consistent with previous findings of *M. luteus* as the single dominant species within this genus. [27, 28] In contrast, while *Mycobacterium* RefSeq genomes have a comparatively low Mash distance range (Supplementary Fig. 11), these distances have been artificially compressed by the overrepresentation of *M. tuberculosis* and other pathogens, as more than 100 species have been sequenced, including six in the NPDC.

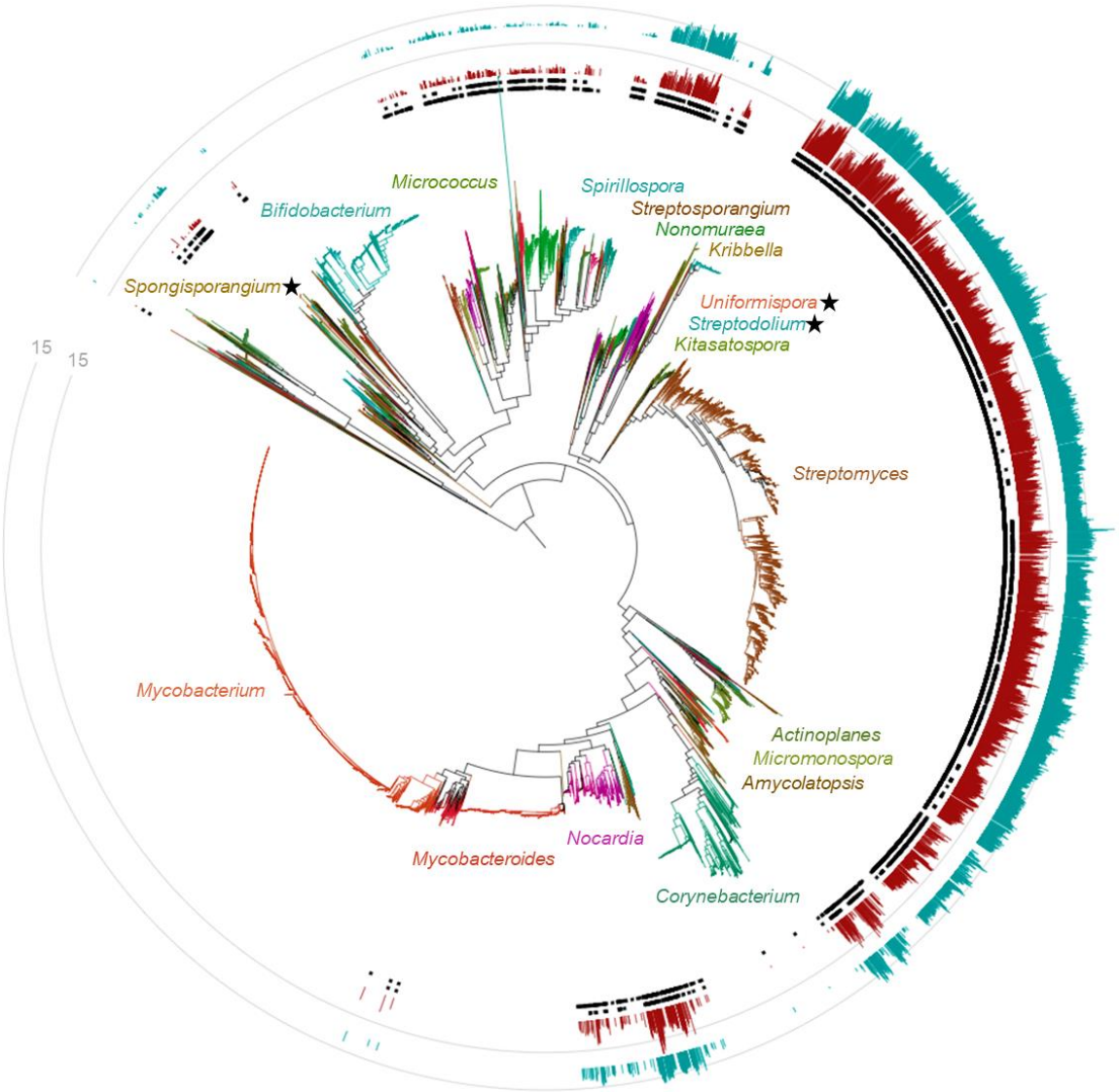

Figure 3. Taxonomic and GCF distribution of NPDC Actinobacteria strains. The genome-based maximum likelihood phylogenetic tree of RefSeq and NPDC Actinobacteria is color-coded by genus, with representative genera labeled. The inner circle of black squares represents NPDC strains. The outer circle of black squares represents new species. The inner red bar chart indicates the number of new GCFs. The outer teal bar chart indicates the number of GCFs that overlap with RefSeq GCFs. New genera identified in this study are indicated with stars.

Within the NPDC Actinobacteria, three putative new genera were identified based on GTDB-Tk and RED values. [24, 29] NPDC049639, herein designated as *Spongisporangium articulatum*, is the sole member of a

new genus within the *Kineosporiaceae* family (RED value 0.829) and is most closely related to *Kineosporia* (Fig. 4A). However, while *Kineosporia* strains have an average genome size of 8.11 Mb, NPDC049639 has a significantly smaller 4.86 Mb genome encoding 11 BGCs. Further differentiating itself from other members of *Kineosporiaceae*, NPDC049639 forms large spherical structures during growth and shows no evidence of motile spores (Fig. 4B and Supplementary Fig. 12). The two other genera, herein named *Streptodolium* and *Uniformispora*, belong to the *Streptomycetaceae* family and are most closely related to *Yinghuangia* and *Embleya*, two genera reclassified from *Streptomyces* in 2018 (Fig. 4C). [30] The two strains of *Streptodolium elevatio*, NPDC002781 and NPDC048946 (RED values 0.843 and 0.844, respectively), share 98.2% genome similarity by ANI, suggesting two strains within a shared species (Supplementary Table 4). [31] NPDC002781 and NPDC048946 have 25 and 29 BGCs in their large 9.46 and 9.59 Mb genomes, respectively. By scanning electron microscopy (SEM), *S. elevatio* NPDC002781 displays long protruding aerial hyphae and barrel-shaped spores (Fig. 4D and Supplementary Fig. 13). In contrast, the two isolates of *Uniformispora flossi*, NPDC059210 and NPDC059280 (RED value 0.843), are likely the same strain (99.98% genome identity by ANI; Supplementary Table 4). [31] *U. flossi* NPDC59280 harbors 24 BGCs within its 8.80 Mb genome, and as indicated by its genus name, its aerial hyphae are long and uniformly segmented, except for nearly spherical terminal spores (Fig. 4E and Supplementary Fig. 14).

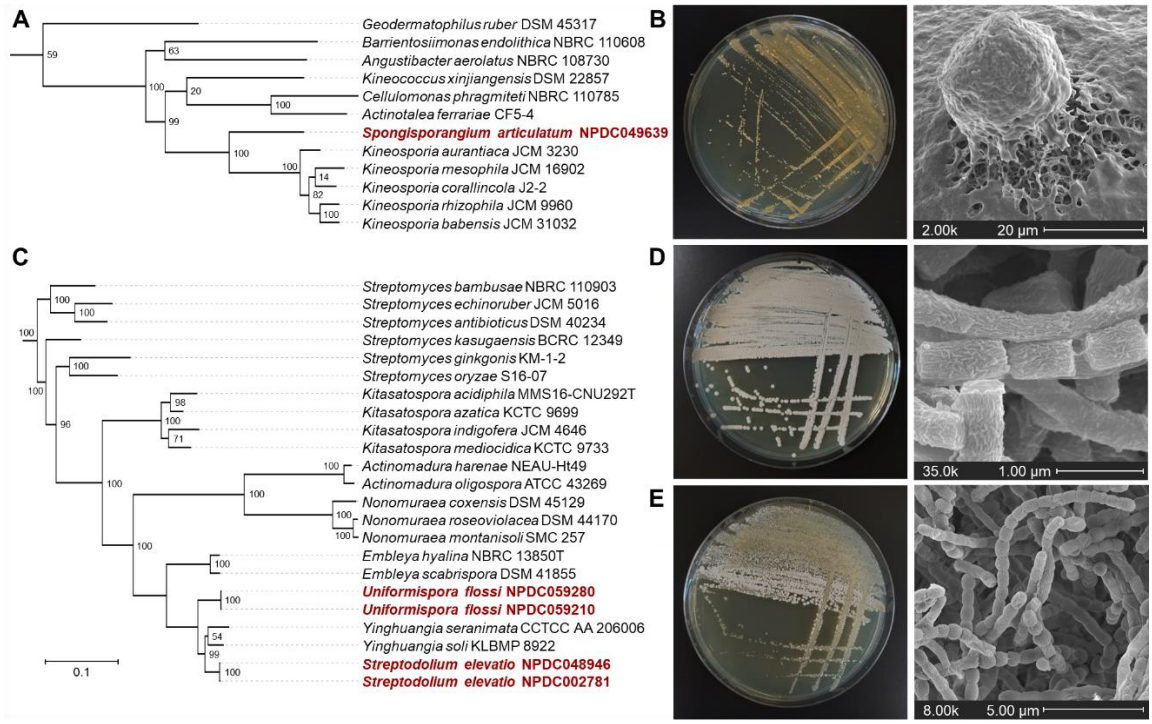

Figure 4. New genera identified in the NPDC. (A) Genome-based maximum likelihood phylogenetic tree showing the position of *Spongisporangium articulatum* NPDC049639 among representative type strains. (B) Representative macroscopic and SEM images of *S. articulatum* NPDC049639 highlighting the large spherical bodies on the spongelike culture surface inspiring the genus name. (C) Genome-based maximum likelihood phylogenetic tree showing the positions of *Streptodolium elevatio* NPDC002781 and NPDC048946 and *Uniformispora flossi* NPDC059210 and NPDC059280 among representative type strains. (D) Representative macroscopic and SEM images of *S. elevatio* NPDC002781 highlighting the fibrous sheath of the aerial hyphae. (E) Representative macroscopic and SEM images of *U. flossi* NPDC059280 highlighting the long chains of uniformly segmented aerial hyphae interconnected with thin filamentous strands. Additional SEM images are found in Supplementary Fig. 12-14.

New microbiology and taxonomic diversity can also be correlated with the biosynthetic diversity and potential of the strains of the NPDC. Actinobacteria typically allocate substantial portions of their genomes

(up to 20%) to BGCs encoding NPs (Extended Data Fig. 2). However, not all genera are equal in their biosynthetic capacities, as might be expected for genera that reside in varying numbers of diverse or isolated ecologies (Supplementary Fig. 15), and an earlier study has shown that host-associated Actinobacteria utilize ~20% less genomic capital for NP biosynthesis than their environmental counterparts. [15] NPDC *Streptomyces* harbor more than 30 BGCs per genome, accounting for 11.7% of their total genomes on average, which is superior to most bacterial genera but falls below 23 other NPDC Actinobacteria genera (Fig. 3 and Extended Data Fig. 2). Many understudied genera such as *Nocardia*, *Actinosynnema*, and the previously highlighted *Embleya*[32] display exceptional biosynthetic potential, with *Embleya* averaging more than 50 BGCs across its ten NPDC genomes. The previous record for highest BGC dedication among Actinobacteria was held by *Kitasatospora kifunensis* DSM 41654 with 26.50% of its genome assigned to BGCs. [15] Within the NPDC, three strains have surpassed this threshold: *Kitasatospora* sp. NPDC008050 (30.32%), *Streptomyces olivoreticuli* NPDC020840 (28.17%), and *Streptomyces klenkii* NPDC049569 (26.51%). In contrast, other genera such as *Rothia*, *Micrococcus*, and *Agromyces* all harbor fewer than five BGCs per NPDC genome. Of the new genera, none of the 11 *Spongisporangium* BGCs share notable similarity to known BGCs available in the MIBiG database, with the most similar BGCs originating from strains in other *Kineosporiaceae* genera or less related genera such as *Mycobacterium*. *Streptodolium* and *Uniformispora*, in contrast, utilize more than 16% of their genomes for secondary metabolism (Extended Data Fig. 2), sharing more in common with the related *Embleya* than the type genus of the family, *Streptomyces*. Compared to *Streptodolium*, *Uniformispora* shares especially little biosynthetic similarity to other known strains.

NPDC Actinobacteria significantly expand the accessible biosynthetic potential.
By collecting and sequencing strains with untapped and exceptional biosynthetic potential, more than 200,000 BGCs have already been identified in NPDC Actinobacteria genomes in this study. Extrapolating this number to encompass the entire NPDC collection based on current sequencing trends suggests the presence of nearly 3.25 million BGCs in total. Grouping of BGCs into GCFs allows for mapping of gene clusters with similar architectures often encoding related molecules and facilitates dereplication of BGCs with known NPs. [33] Additionally, while most NPDC BGCs are predicted by antiSMASH to be intact (Extended Data Fig. 3A), GCFs have the advantage of grouping the remaining fragmented BGCs together, enabling identification of otherwise missed producers of a desired NP type (Extended Data Fig. 3B).

Comparison between RefSeq and NPDC Actinobacteria GCFs shows that the rate of GCF discovery directly correlates to the increase in Actinobacteria genomes. A total of 20,112 GCFs were extracted from the 21,186 RefSeq Actinobacteria, and the 7142 NPDC Actinobacteria harbor 16,902 GCFs. With more than 40% of the identified GCFs being unique to the NPDC, release of these genomes results in ~25% increases of both publicly available genomes and GCFs (Fig. 5A). More than 85% of the NPDC GCFs are found exclusively in only a single genus, including most of the GCFs found in RefSeq and NPDC. Further, 36% of NPDC GCFs are unique to individual strains (Extended Data Fig. 4A). In contrast, the most conserved GCFs in most Actinobacteria genera are found in over 90% of NPDC genomes (Extended Data Fig. 4B).

Out of the 16,902 GCFs in NPDC genomes, most are expected to represent unexplored chemical space (Supplementary Fig. 16). Only 454 GCFs contain characterized BGCs from the MIBiG BGC database—including 28 GCFs with MIBiG entries lacking available genomes for the respective strains (Fig. 5A and Supplementary Table 5). [34] Nearly 12,000 total GCFs and 5000 non-RefSeq GCFs originate from NPDC *Streptomyces* strains, in line with the overall breakdown of NPDC GCFs (Fig. 5B). As with taxonomic diversity, not all genera show similarly high percentages of non-RefSeq GCFs. All genera will eventually reach a plateau at which they will experience diminishing rates of GCF novelty by genome sequencing, but the rate at which they will reach that plateau varies significantly. For example, the phylogenetically uniform and BGC-poor *Micrococcus* does not harbor any non-RefSeq GCFs despite dozens of NPDC genomes (Fig. 5B). Many prominent genera, such as *Streptomyces*, *Nocardia*, and *Actinoplanes*, well-regarded for their biosynthetic potential and with high taxonomic diversity (Fig. 2), exhibit between 40-50% non-RefSeq GCFs (Fig. 5B), higher than most non-Actinobacteria genera. [6] In many of the rarer, underexplored Actinobacteria genera such as *Umezawaea* (58.1%), *Microbispora* (60.2%), and *Actinosynnema* (59.2%),

this trend is even more pronounced, emphasizing them as valuable targets for future genome sequencing with their plateaus approaching significantly slower than in the well-studied genera (Supplementary Fig. 17).

While the level of GCF novelty varies by genus, the overall trend only slightly favors new species (42.0% non-RefSeq GCFs) over known species (41.1% non-RefSeq GCFs) among NPDC genomes (Fig. 5C). This small difference is unsurprising as more than 80% of NPDC GCFs are found in ten or fewer genomes (Extended Data Fig. 4), and most GCFs are strain- or species-specific.[6] This trend is also reflected by more than half of NPDC BGCs, from both GTDB known and new species, displaying little similarity to characterized BGCs in the MIBiG database (Fig. 5D).[34] Many genera or species, especially rare ones, have adapted to specific ecological niches through the acquisition and evolution of tailored BGCs. In particular, Actinobacteria from the soil tend to have evolved more niche-specific GCFs, with two-thirds of GCFs identified in the genomes of NPDC Actinobacteria isolated from soil not being found in other ecologies (Supplementary Fig. 15B). This trend is more pronounced in certain rare genera, such as *Actinoplanes* (44.9% versus 31.3%), in which new species harbor far more non-RefSeq GCFs per strain than known species (Fig. 5C, Supplementary Fig. 18).

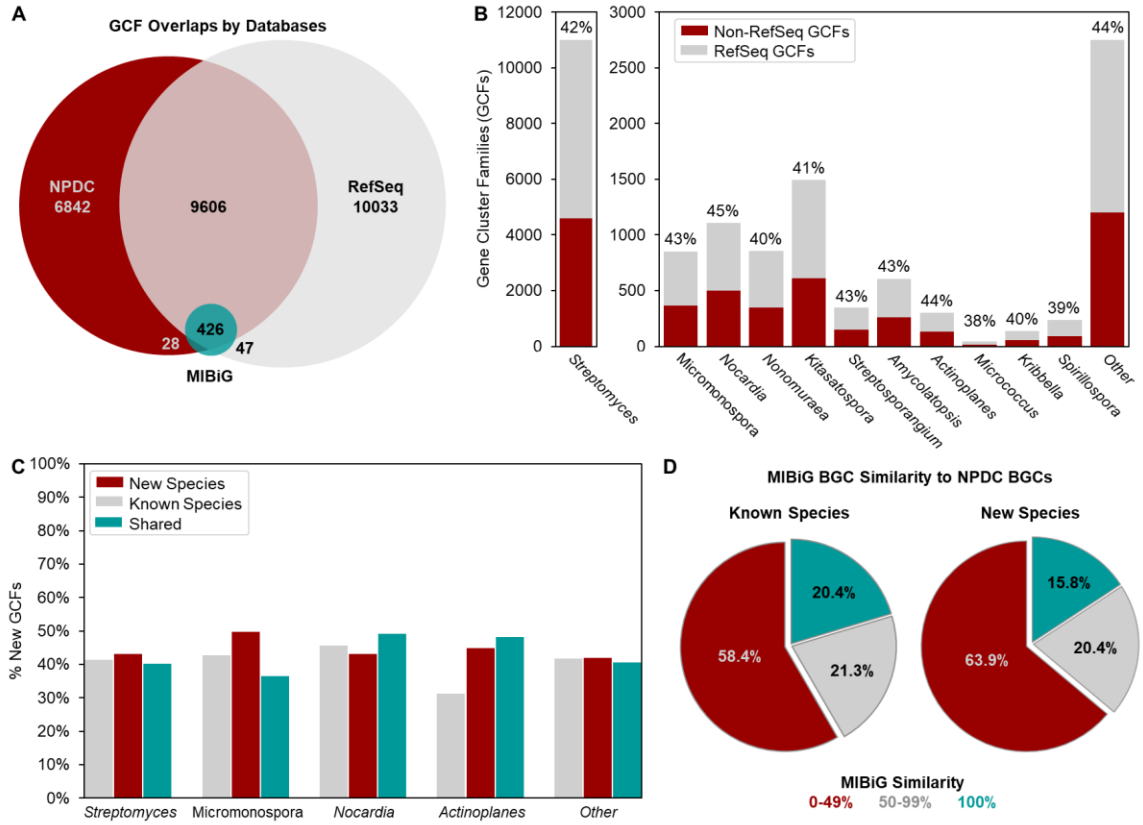

Figure 5. The NPDC genomes expand the accessible biosynthetic potential across different genera. (A) Distribution of GCFs in RefSeq (grey) and NPDC (red) genomes, with Actinobacteria MIBiG GCFs (teal) included separately. (B) Distribution of non-RefSeq GCFs (red) and GCFs observed in both NPDC and RefSeq genomes (grey) across representative genera as visualized in a stacked bar chart. Between 40-50% of total GCFs by NPDC genus are not observed in RefSeq (indicated by percentage). Also see Supplementary Fig. 17. (C) Percentages of non-RefSeq GCFs in known species (grey), new species (red), and shared between them (teal) across representative genera. Also see Supplementary Fig. 18. (D) Distribution of NPDC BGCs by antiSMASH-assigned similarity to characterized MIBiG BGCs. A higher percentage of BGCs with low similarity to MIBiG BGCs is observed for new species relative to known ones.

The NPDC Portal enables virtual and physical access to the NPDC strain collection. The combined phylogenetic and biosynthetic diversity of the NPDC strains underlines its value to the research community, but its full potential can only be realized by making both the genomes and physical strains accessible. We have created an online portal (npdc.rc.ufl.edu/home) as a central hub for making this growing and incomparable resource accessible to enable users worldwide to take advantage of its unique resources. The NPDC Portal is centered around three primary functions: (i) strain and genome databases, (ii) integrated bioinformatics analyses, and (iii) strain requests for physical experimentation (Supplementary Fig. 19, Extended Data Fig. 5).

Many of the NPDC strains have metadata associated with them, so in addition to the genomes, the NPDC Portal contains any associated collection information (ecology, date, and location), media the strain has been grown in, genome-based taxonomy including related strains, and strain pictures (Supplementary Fig. 20). [5] Additionally, antiSMASH-generated BGCs are available for download or online browsing, and BGC annotations are provided for all genomes (Supplementary Fig. 21). The built-in customized DIAMOND-BLASTP tool can be used to query protein databases encompassing all NPDC genomes or BGCs. [35] The NPDC BGC database, containing more than 224,000 BGCs, can be queried directly, with the added option to use up to five sequences simultaneously to explore co-occurrence within individual BGCs (Supplementary Fig. 22). The BLASTP results can be downloaded in various formats, including a standard BLAST tabular result, a multiFASTA file containing all protein hits, and a multiFASTA file containing all protein sequences from BGCs encoding hits. The multiFASTA files directly provide the sequences needed to construct sequence similarity networks (SSNs) or genome neighborhood networks (GNNs) using established tools such as EFI, which can be powerful for biosynthetic studies or prioritization of BGCs for discovery. [36] The BGC BLASTP results are also tied to NPDC GCF numbers and to antiSMASH-generated MIBiG BGC assignments, so related hits can be quickly extrapolated from a single BLASTP search.

Applications of the NPDC Portal to explore the biosynthetic potential of the collection.

To highlight the potential of the NPDC Portal, we have chosen four examples to emphasize possibilities for mining the BGC database with BLASTP (A), identifying associated multi-gene sets using BLASTP (B), and generating outputs to build SSNs (C) and GNNs (D; Fig. 6). Our chosen examples cover resistance gene mining for the discovery of novel antibiotics, scaffold-specific mining for NPs to diversify the characterized chemical space, prioritization of enzymes for exploitation as biocatalysts, and mapping of biosynthetic proteins from a given NP family to study divergent biosynthetic chemistry, resistance, or regulation.

The NPDC BGC BLASTP database enables a user to search directly for protein hits within BGCs, which is especially advantageous to distinguish between primary metabolism proteins and those that have evolved into NP-specific roles. In the specific case of antibiotic resistance genes, this differentiation is particularly helpful as a second copy encoding a mutated target protein is often localized within the BGC encoding the antibiotic NP to confer resistance to the producing organism. [17] Resistance gene-guided genome mining can therefore be used to prioritize BGCs for NPs with specific targets such as DnaN, the beta subunit of the DNA polymerase III. DnaN has been previously validated as an attractive novel antibacterial target; however, griselimycin, with its resistance protein GriR, remains the only DnaN-targeting NP to date (Fig. 6A). [37] To identify other BGCs encoding NPs potentially targeting DnaN, we queried the NPDC BGC database for GriR homologues. After inspection for conservation across GCFs and for dissimilarity to primary metabolism DnaN copies, at least 14 BGCs across four GCFs from *Streptomyces* and *Kitasatospora*, each distinct from griselimycin, could be prioritized for ongoing follow-up studies (Fig. 6A and Supplementary Fig. 23). Some of these BGCs can be found in only NPDC genomes; however, this tool has identified other BGCs that are present in RefSeq genomes but had not previously been investigated for DnaN-targeting activity of the encoded NPs.

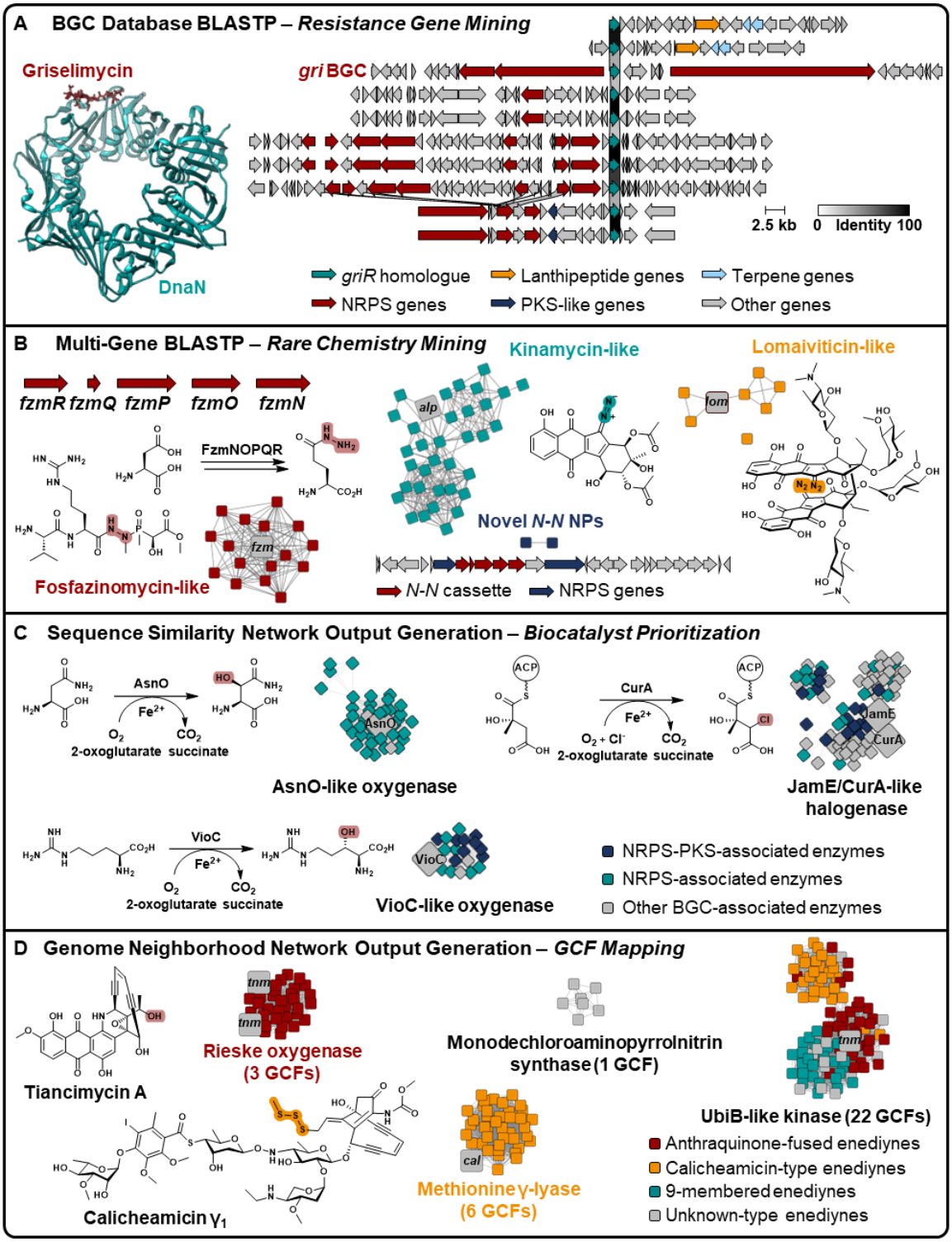

Figure 6. Genome mining using the NPDC Portal. (A) The DIAMOND-BLASTP tool allows querying of the BGC database allowing exclusion of most primary metabolism hits. Inspired by griselimycin, the only known DnaN-targeting NP to date and shown bound to DnaN (PDB: 6PTV), NPDC BGCs encoding DnaN homologues were identified, and members from four GCFs were visualized using Clinker.[37, 38] (B) The NPDC Portal enables up to five simultaneous BLAST queries. As an example, the five gene cassette

required for *N-N* bond formation in fosfazinomycin was queried against the NPDC BGC database. The resultant BGCs were clustered by BiG-SCAPE and assigned to known and unknown NP families.[39] For the unknown family of BGCs containing this five-gene cassette, a representative BGC map is depicted. Also see Supplementary Fig. 24. (C) In searching for potential biocatalysts, BGC-associated α-KG-dependent dioxygenases were identified with the NPDC BLASTP tool, grouped using the EFI SSN tool, and color-coded using the NPDC-provided and antiSMASH-generated BGC classes.[36] Also see Supplementary Fig. 25. (D) Using combined BLASTP queries for TnmE and TnmE4 from tiancimycin A biosynthesis, all protein sequences encoded by NPDC enediyne BGCs were downloaded and grouped using the EFI SSN tool.[36] Nodes were color-coded based on known and unknown enediyne subclasses in an effort to identify potential new chemistries and scaffolds. Also see Supplementary Fig. 26 and 27.

In a related approach, the NPDC BGC database and its associated BLASTP tool facilitate the discovery of BGCs responsible for complex biosynthetic transformations or installation of multiple pharmacophores involving up to five proteins. Often, structural complexity is introduced by multi-enzyme machineries, and searching for individual instances of proteins is insufficient for identifying BGCs in which they co-occur. To demonstrate the efficacy of this approach, the five proteins responsible for introduction of an uncommon *N-N* bond in the antifungal fosfazinomycin via a glutamylhydrazine intermediate (FzmN-R) were simultaneously used to query NPDC BGCs (Fig. 6B and Supplementary Fig. 24).[40] By downloading the hit BGCs and clustering them using the external tool BiG-SCAPE, four NP families could be grouped together, including fosfazinomycin-like, kinamycin-like, and lomaiviticin-like NPs (Fig. 6B).[39] While some encoding strains represent alternative producers, many others represent novel variations, as well as a predicted novel *N-N* bond-containing NP scaffold without notable similarity to BGCs in the MIBiG database.

Beyond the discovery of NPs, the NPDC also facilitates the discovery of enzymes for use as biocatalysts. As above, searching the BGC database directly focuses the search on non-primary metabolism hits, and the accompanying downloadable BGC metadata file provides the BGC class for each hit. In combination with external tools such as the EFI web-based SSN tool, the enzyme hits can be mapped to putative functions or substrates. In an example, the NPDC was mined for BGC-associated α-ketoglutarate-dependent dioxygenases and halogenases, the generated 'protein hits multiFASTA' imported into the EFI web-based SSN tool, and the resulting network explored using Cytoscape (Fig. 6C and Supplementary Fig. 25).[36, 41] Upon enrichment with GCF, taxonomy, and BGC class metadata, dozens of enzymes responsible for modification of amino acids as substrates for co-encoded non-ribosomal peptide synthetases (NRPSs; VioC- or AsnO-like oxygenases), halogenation of carrier protein-tethered polyketide intermediates (CurA-like halogenases), or oxidation of many other diverse substrates could be rapidly categorized.[42]

Analogous to identifying related enzymes, the NPDC BLASTP tool can deliver metadata and sequences for all proteins from related BGCs. In combination with the EFI SSN tool, large-scale comparison can be performed for thousands of individual proteins encoded by GCFs related by core genes.[36] In doing so, trends that are not readily apparent from smaller BGC alignments may be identified. One NP family where this analysis can provide valuable biosynthetic insights is the enediyne family of NPs. The enediynes, structurally related by their DNA-damaging core containing an alkene flanked by two alkynes, display notable biosynthetic variety across their more than 1000 NPDC BGCs, while still identifiable by querying with TnmE (enediyne polyketide synthase, PKS) and TnmE4 (conserved enediyne biosynthetic protein) from tiancimycin A biosynthesis (Fig. 6D, Supplementary Fig. 26 and 27).[43, 44] Because of their structural and taxonomic diversity, related enediynes and their BGCs do not always cluster well when using whole-BGC methods such as BiG-SCAPE. Instead, specific proteins responsible for conserved moieties, such as the methyltrisulfide in calicheamicin-like enediynes, can be used to classify different BGC subclasses (Fig. 6D). Confidence in these subclasses can be strengthened by mapping the GCF assignments onto the SSN (Supplementary Fig. 27). SSN clusters comprised of proteins from many GCFs are likely to be core natural product scaffold biosynthetic enzymes or non-biosynthetic proteins, while SSNs clusters that are associated with a smaller number of GCFs are more likely to be biosynthetic tailoring enzymes.

Discussion

The NPDC marks a transformative initiative by tapping into the vast biosynthetic potential of Actinobacteria for NP discovery. With over 122,000 strains, of which more than 84% are expected to be Actinobacteria, the NPDC collection represents a repository rich in genomic diversity. The analysis of the first 8,490 released genomes reveals the expansive taxonomic range within Actinobacteria, underscoring the significance of genome sequencing in preserving and exploring biodiversity.

One key aspect of genome sequencing is the reliable taxonomic classification of the strains in the collection, which significantly increases the value of the collection for microbiological and biosynthetic studies as there is a notable discrepancy between the phenotype- and genome-based taxonomic assignment. For many rare genera, this disparity can be explained by the evolving nature of taxonomic nomenclature or the close relatedness between genera. For example, among the 86 strains assigned as *Actinomadura*, nearly half can now be assigned as *Nonomuraea*, a genus that was not defined until the 1990s. [45] Others belong to *Spirillospora*, a genus whose members share very high 16S rRNA sequence similarity to some *Actinomadura* strains. [46] The updated taxonomies thus emphasize the critical importance of genome sequencing and the preservation of the genomic diversity encapsulated within the NPDC collection. Despite the differences between the phenotype- and genome-based taxonomies, this survey confirms that the NPDC collection has successfully prioritized environmental over health-associated Actinobacteria and therefore is complementary to existing genomes in RefSeq. Overall, the untapped diversity awaiting exploration within many rarer genera stored in the NPDC collection emphasizes the need for further sequencing and cataloguing efforts. By prioritizing these genera based on their observed intrageneric diversity, we can shed more light on this crucial branch of the tree of life while identifying promising candidates for future discovery campaigns. Within ~7% of the NPDC collection, three new genera could be identified, serving as compelling examples of the microbiological discoveries possible through the NPDC and as inspiration for future studies enabled by the NPDC collection. However, while the microbiological facet of the NPDC holds substantial value, its greatest strength arguably resides in the biosynthetic machineries encoded by these strains.

Consistent with previous studies emphasizing the value of taxonomic distance for NP discovery, [6] the percentage of antiSMASH-annotated BGCs with 100% identity to MIBiG BGCs is lower in new species compared to known species (Fig. 5D), but there is only a weak correlation with taxonomic novelty at the genus level (Fig. 5B-C). Instead, we observe that biosynthetic specialization occurs mostly at the individual strain level, as more than 80% of all GCFs are found in fewer than 10 strains (Extended Data Fig. 4A). With more than 200,000 BGCs assigned in the initial NPDC genome release, prioritization of BGCs of interest and determination of the rate of return on sequencing for novel biosynthesis require grouping into GCFs. As structural diversity often is introduced by downstream modifications using single enzymes, grouping into GCFs is not a perfect measurement for NP type and scaffolds, but it does provide an approximation of the diversity of the BGCs and, to a lesser degree, NPs. Most of the GCFs that occur across species and genera often encode NPs such as siderophores or other common metabolites, e.g., geosmin, that are often critical for a strain's survival independent of most environmental factors. [47, 48] Outside of these highly prevalent GCFs however, the 5% cut-off utilized to define distinct species provides sufficient genomic space for harboring new BGCs even within known species. For the median NPDC Actinobacteria genome, 5% is equivalent to more than 400 kb or twelve average-sized BGCs (33 kb in NPDC genomes). This observation is in line with the relatively consistent levels of biosynthetic novelty observed across Actinobacteria genera (Fig. 5B) by assuming a stable set of core GCFs and a more variable set of GCFs for rapid ecological adaptation. However, the argument for sequencing beyond known species and *Streptomyces* remains, as it is still rare for these non-core GCFs to cross between genera. [6]

While the cost of genome sequencing has been dropping dramatically in recent years, it is still not considered cost- nor time-efficient for every lab to sequence all strains that have been collected—leading to sequencing primarily of individual pre-selected strains. Herein, we have shown the power of the approach of sequencing at scale, without being biased towards niche uses but also having the genotype-phenotype linkage lacking in large-scale metagenomic sequencing efforts. Our results underpin that broad sequencing efforts are especially invaluable for studying less prevalent GCFs that will otherwise be missed. The presented online NPDC Portal relies on making robust and established methods easily applicable to this

large dataset for expert and non-expert users alike, with the added benefit of increasing use of valuable strains that might otherwise stay hidden away, unsequenced, in a freezer in an individual lab. As maximizing the value of this resource goes beyond any single research group, our goal is that by (i) enabling queries previously inaccessible to non-computational researchers and (ii) making available strains that are prioritized by these queries, we can democratize genome mining for NP chemists and other non-specialists. Our presented resistance gene mining example (Fig. 6A) shows that, while not replacing existing resistance-guided tools such as ARTS, [49] resistance gene-mining via the NPDC Portal offers a rapid survey of NPDC genomes without an advanced bioinformatics background and avoids the pitfall of a standard BLAST database that will return mostly primary metabolism hits. The scope of this tool also goes far beyond resistance-guided genome mining, as highlighted by each of the remaining examples. The multi-BLAST function could, in addition to the presented example for mining *N-N* containing NPs (Fig. 6B), be applied to many other strategies, such as looking for specific combinations of NRPS and PKS domains or biosynthetic enzymes responsible for installation of one or more pharmacophores or functional groups. For synthetic chemists, the access to a wide range of strains encoding a selection of potential biocatalysts (Fig. 6C) allows circumvention of issues with stability, solubility, promiscuity, or other characteristics without resorting to enzyme engineering. Further, the ability to map biosynthetic proteins associated by a conserved chemical moiety or scaffold by GCF membership (Fig. 6D) enables researchers to draw conclusions on the relative importance of specific proteins that might otherwise go unnoticed through examination of hundreds of BGC sequences or unbiased collections of protein sequences from large genome datasets. Application of this tool to any set of BGCs related by one or more genes therefore provides a more holistic view of BGC diversification, especially in larger NP families.

With so many uncharacterized GCFs and low overlap with BGCs from the public databases, NPDC strains encode vast potential for NP and enzyme discovery. The NPDC Portal serves as a gateway to these resources, facilitating access to strains and associated metadata, while offering a suite of tools to expedite the functional or fundamental exploration of BGCs. By democratizing access to this wealth of strains and genomic data, the NPDC is positioned to accelerate advancements in drug discovery, biocatalysis, microbiology, and biotechnology.

Materials and Methods

General experimental procedures, bacterial strains, and chemicals

All 8490 strains were obtained from the Natural Products Discovery Center (NPDC) strain collection located at The Herbert Wertheim UF Scripps Institute for Biomedical Innovation & Technology in Jupiter, Florida. All strains were originally stored as either frozen glycerol spore stocks or lyophilized stocks. Most strains were cultured for 7 days in 5 mL modified tryptic soy broth supplemented with optimized levels of trace elements (TSB+): $ZnCl_2$ (40 mg L^{-1}), $FeCl_3 \cdot 6H_2O$ (200 mg L^{-1}), $CuCl_2 \cdot 2H_2O$ (10 mg L^{-1}), $MnCl_2 \cdot 4H_2O$ (10 mg L^{-1}), $Na_2B_4O_7 \cdot 10H_2O$ (10 mg L^{-1}), and $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (10 mg L^{-1}). [50] When strains were grown on solid media, either ISP2 or ISP4 media were used. [51] Solid and liquid media were supplemented with nalidixic acid (30 mg L^{-1}) and cycloheximide (50 mg L^{-1}).

DNA concentrations were determined fluorometrically using the Quant-iT Broad Range and High Sensitivity dsDNA assays (Invitrogen) on an infinite M1000Pro plate reader (Tecan). DNA quality was determined using a NanoDrop 2000C spectrophotometer (Thermo Scientific).

The Lotus library preparation kits were purchased from Integrated DNA Technologies (IDT). The Agencourt AMPure XP-PCR purification beads were purchased from Beckman-Coulter. All solvents (molecular biology grade) or chemicals were purchased from standard commercial sources.

Genomic DNA isolation

To isolate gDNA from bacterial cultures, samples were processed in batches of 96 cell pellets dissolved in 740 µL TE buffer (original dilutions based on culture density). Cells were lysed using 20 µL lysozyme (100 mg mL^{-1}) and 10 µL achromopeptidase (5 mg mL^{-1}) in the presence of 5 µL RNase A (20 mg mL^{-1}) at 37 °C

for 2 h with periodic inversions. Next, 40 µL sodium dodecyl sulfate (10% w/v) and 10 µL proteinase K (8 mg mL^{-1}) were added, mixed, and incubated at 56 °C for 16-24 h until samples were clear. 100 µL NaCl (5 M) was then added to the samples and mixed, followed by the addition of 500 µL 25:24:1 phenol:chloroform:isoamyl alcohol. Centrifugal phase separation (10 min at 13.2k rpm) was aided by the addition of 75 µL Molykote high vacuum grease (DuPont). A second extraction was performed using 500 µL 24:1 chloroform:isoamyl alcohol and separated by centrifugation (10 min at 13.2k rpm). A final ethanol precipitation with sodium acetate (final concentration of 0.3 M) was performed, and the final gDNAs were resuspended with 100 µL low-EDTA TE buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA). gDNA qualities were determined spectrophotometrically, and gDNA concentrations were determined fluorometrically.

Genome sequencing, assembly, and annotation

Most library preparations for short-read sequencing were carried out using IDT Lotus reagents with a modified automated protocol on an Agilent Bravo liquid handling robot. The manufacturer's instructions were utilized at a quarter scale except for the final bead purification and elution step, which was conducted at full volume. The libraries were PCR-amplified. Each library was indexed with IDT UDI 10 bp barcodes and pooled in batches of 384 normalized samples each. Genomes were sequenced using 2x150 sequencing on an Illumina NovaSeq 6000 with an S4 flow cell (384 samples per lane).

To process sequencing reads, BBDuk was used to remove contaminants, trim reads that contained adapter sequence and terminal homopolymers of five or more 'G' bases, and remove reads containing one or more 'N' bases or having length ≤ 51 bp or 33% of the full read length. [52] Reads mapped with BBMap to masked human references at 93% identity were separated into a chaff file. [52] Further, reads aligned to masked common microbial contaminants were separated into a chaff file.

The following steps were performed for genome assembly: (1) artifact-filtered and normalized Illumina reads were assembled with SPAdes (version v3.14.1; –phred-offset 33 –cov-cutoff auto -t 16 -m 64 –careful -k 25,55,95); (2) contigs were discarded if the length was < 1 kb (BBTools reformat.sh: minlength=1000 ow=t). [52, 53] CheckM then was used to calculate the contamination and completeness level of genomes. [54] Genomes having ≥ 95% completeness and ≤ 10% contamination were kept, while others are discarded. GTDB-Toolkit was used to annotate the taxonomy of genomes. [24] Prokka was used to predict and annotate coding sequences in the genomes. [55]

Construction of NPDC Portal

The NPDC Portal is written in Python 3.11 using Flask (v2.3) and Jinja2 (v3.1) libraries. The web server is linked to three separate SQLite databases for storing genomic, users, and BLAST query data. The BLAST function is provided via a separate Python script running in the background, calling DIAMOND-BLASTP (v2.0.15) on a pre-built NPDC protein database (default sensitivity settings with modified query parameters: e-value = $1e^{-10}$, query-cover = 80%, identity-threshold = 40%).[35] Raw text results from DIAMOND were then parsed and stored accordingly in the query database to facilitate interactive viewing and downloading. The source code for the NPDC Portal is available at https://github.com/shenlabnp/npdc_portal.

Bioinformatics analysis

Assembled genome data were downloaded in GenBank format (.gbk) from the NCBI RefSeq FTP server using the ncbi-genome-download tool (April 2022). [56] The list of RefSeq accessions was cross-referenced with the GTDB taxonomy RS-207 (April 2022) to get the whole-genome-based taxonomic classification of each genome. [16, 24] NCBI RefSeq genomes without any GTDB annotation (i.e., more recent and low-quality genomes) were filtered out, leaving a final list of 215,240 genomes. These genomes were selected due to their high quality, enabling a useful comparative BGC analysis, and due to being included in the current version of GTDB, enabling a useful taxonomic comparison.

Assembled NPDC genomes were processed using the GTDB-TK pipeline (v2.1.0) to assign taxonomy based on the same GTDB RS-207 database. [24] Additionally, an internal taxonomic grouping was performed by calculating pairwise ANI-like distances between NPDC genomes using Mash (v2.3). [26] Then, a complete-linkage hierarchical clustering was run with a threshold value of 0.05 (roughly equivalent to 95% ANI value,

a general cutoff for species demarcation.[57] Finally, the genomes were categorized into "known species" for genomes successfully annotated by GTDB down to the species level and "new species" for genomes without corresponding GTDB species annotation. The Mash-derived genome clusters were used to assign putative species grouping to all genomes categorized as the latter.

AntiSMASH (v5.1.1) was run for each genome from NPDC and NCBI RefSeq datasets to identify BGCs.[58] The two datasets were complemented by another set of 1910 experimentally-validated BGCs from the MIBiG v2.0 database.[34] All GenBank BGC files were run using a modified version of BiG-SLiCE which uses cosine-based distance to enable accurate BGC clustering.[59] A cosine distance threshold of 0.4 was used to group BGCs into GCFs. The absence and presence of GCFs across the three datasets could then be tracked, with a GCF determined to be present in a dataset if at least one member BGC is from the given dataset.

To create the phylogenetic trees, the GTDB-Tk toolkit was utilized using the identify and align commands with combinations of NPDC and RefSeq genomes.[24] The GTDB-Tk outputs were converted into phylogenetic trees using fasttree and visualized on iTOL.[60, 61] For further taxonomy-based analyses, genera grouped in GTDB under the same parent name but considered different genera (e.g., *Arthrobacter*, *Arthrobacter_C*) were compared together to be consistent with the taxonomic assignments provided in RefSeq (Supplementary Table 6).

Genome mining

For DnaN-encoding BGC mining, the amino acid sequence of GriR encoded by *Streptomyces muensis* was used as the query sequence for the NPDC BGC Database (sequence retrieved from the griselimycin BGC; MIBiG BGC0001414).[34, 37] For each GCF associated with a NPDC BLASTP hit, the DnaN homologue was manually compared with any other homologues encoded by the same genome (if any) and DnaN copies not associated with BGCs from related strains. All antiSMASH-annotated BGCs encoding divergent DnaN homologues were downloaded as .gbk files from the NPDC Portal and aligned with the griselimycin BGC (MIBiG BGC0001414) using Clinker.[38]

For mining for *N-N* bond-encoding BGCs, the five proteins responsible for installation of the *N-N* bond in fosfazinomycin biosynthesis (FzmN/FzmO/FzmP/FzmQ/FzmR) were used as simultaneous queries of the NPDC BGC Database (sequences retrieved from the *Streptomyces* sp. WM6372 fosfazinomycin BGC; MIBiG BGC0000937).[34, 40] All BLASTP BGC hits were downloaded as .gbk files from the NPDC Portal and clustered using BiG-SCAPE with standard parameters and visualized using Cytoscape.[39, 41] BGC sequences from each BiG-SCAPE cluster were aligned together with the fosfazinomycin BGC and either the *Streptomyces murayamaensis* kinamycin BGC (MIBiG BGC0000236) or the *Salinispora pacifica* lomaiviticin BGC (MIBiG BGC0000240) using Clinker.[38, 40, 62] For the example of searching for BGCs that also encoded a phosphonate NPs, the protein FzmC from the fosfazinomycin BGC was used in conjunction with FzmN/FzmO/FzmP/FzmR to query the NPDC BGC Database.

For mining α-ketoglutarate-dependent dioxygenases, two methods were used. In the first method, individual sequences (VioC, AsnO, and CurA) were used as individual queries for the NPDC BGC Database.[42] The resulting multiFASTA outputs were downloaded from the NPDC Portal and submitted to the EFI SSN tool using an e-value of e^{-150} and visualized using Cytoscape.[36, 41] The resulting SSN was color-coded in Cytoscape using antiSMASH-assigned BGC class using the information from the associated metadata file obtained when downloading the multiFASTA. In the second method, used to showcase the diversity of all α-ketoglutarate-dependent dioxygenase diversity in the NPDC, a multiFASTA containing all BGC-associated Cupin family sequences were obtained using a custom in-house script using the HMM models for the 69 Pfam families belonging to the Cupin clan (CL0029).[63] The multiFASTA output was treated in the same way as in the first method, resulting in Supplementary Fig. 25.

For mining enediyne BGCs, two conserved core enediyne proteins, TnmE and TnmE4, from the tiancimycin A BGC (MIBiG BGC0001378) were used as simultaneous queries for the NPDC BGC Database.[43, 44] The resulting BGC hits multiFASTA file was downloaded, containing the sequences of all proteins encoded by

any BGC containing homologues of both TnmE and TnmE4. The multiFASTA file was uploaded to the EFI SSN tool using an e-value of e^{-100} and visualized in Cytoscape. [36, 41] The resulting SSN was color-coded in Cytoscape using a presence-absence map of key conserved proteins associated with either calicheamicin-type enediynes, anthraquinone-fused enediynes, or 9-membered enediynes.

Electron microscopy

Colonies were fixed in 4% paraformaldehyde and 2.5% glutaraldehyde in 1X PBS, containing 0.1% Triton X-100 pH: 7.2. Fixed cells were processed in 78 µm microporous specimen capsules (EMS) and with the aid of a Pelco BioWave laboratory microwave (Ted Pella). Fixed colonies were washed with 1X PBS, pH 7.22, post fixed with buffered 2% OsO4, water washed and dehydrated in a graded ethanol series 25% through 100%, with 10% increments. Samples were critical point dried using the Tousimis Autosamdri-815 (Tousimis). Once dried, the samples were mounted onto a 12 mm Carbon Conductive Adhesive Tab and aluminum stub (EMS) and then sputter coated with gold/palladium under argon gas, using the Denton Desk V sputter coater (Denton Vacuum). Samples were imaged with Hitachi SU-5000 FE-SEM (Hitachi High Technologies in America) with use of EM Wizard software.

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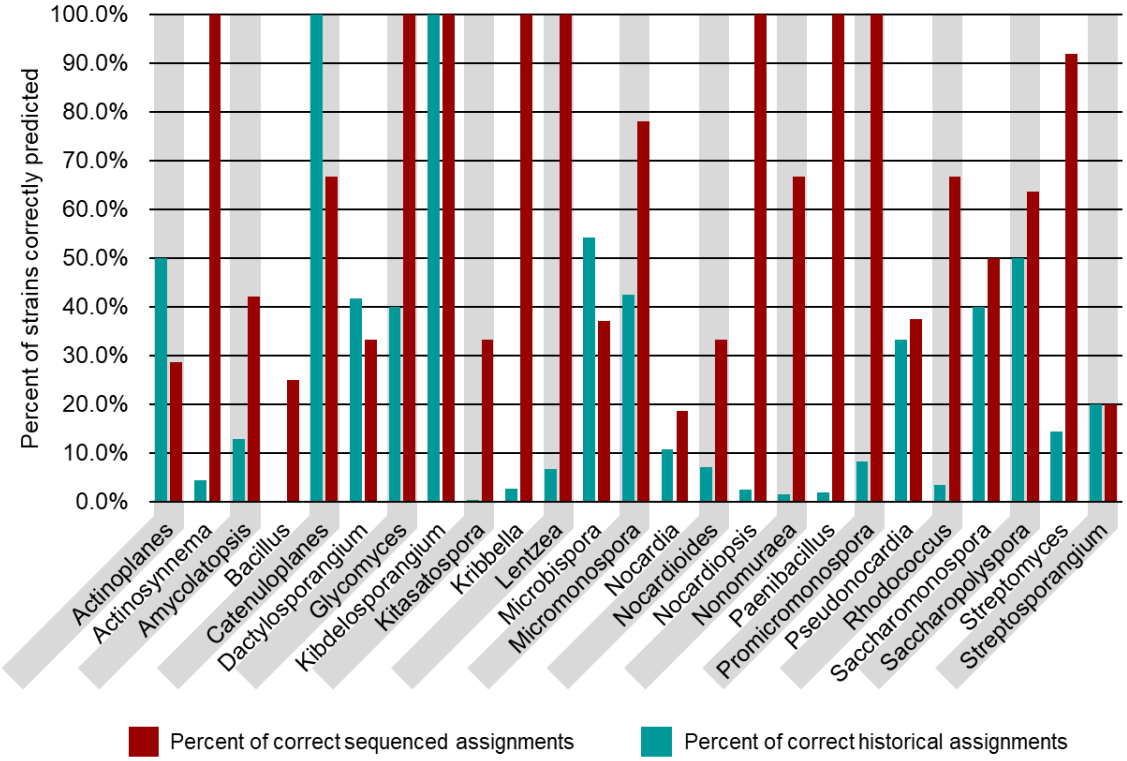
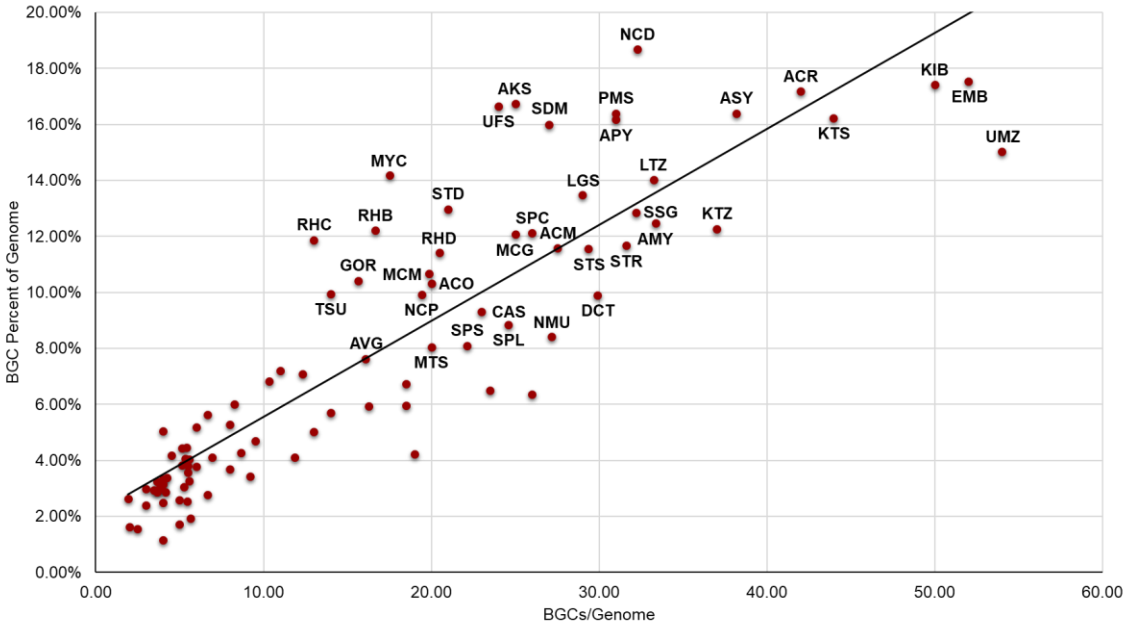
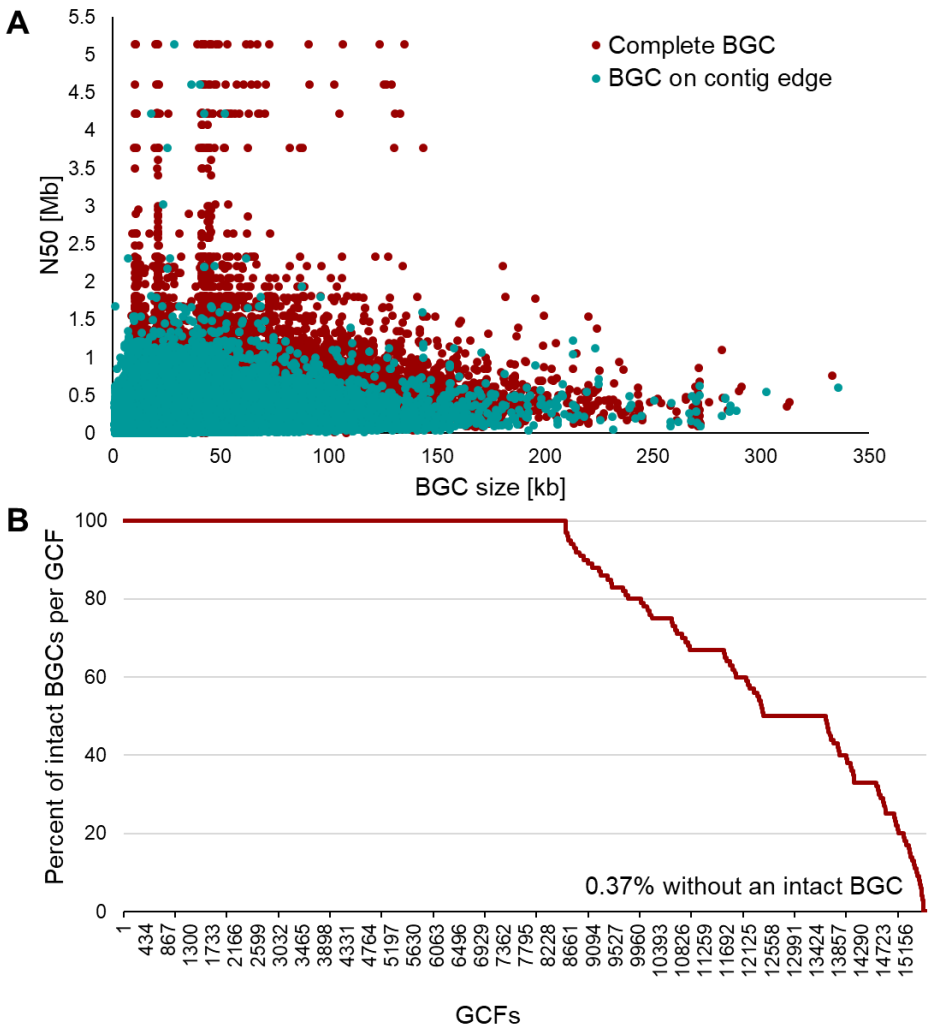
Extended Data Figures



Extended Data Fig. 1. Comparison of historical and sequence-based taxonomies of NPDC strains. Approximately 10% of NPDC strains had morphology-based taxonomies assigned. See Supplementary Table 3 for additional values.

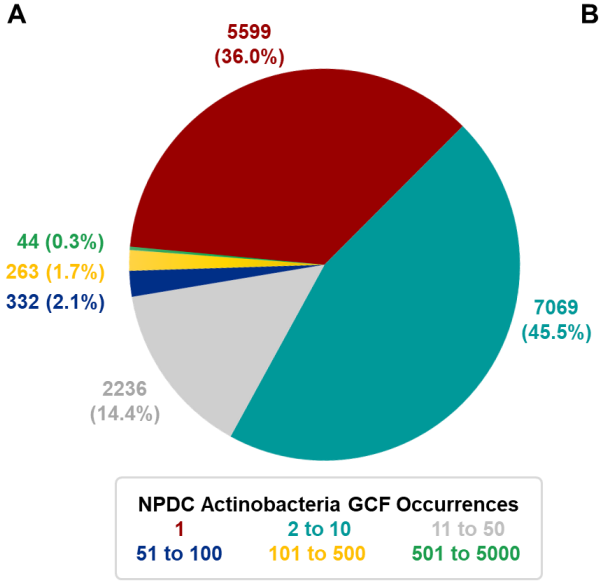


Extended Data Fig. 2. BGC dedication by Actinobacteria genus. The number of BGCs per genome follows a roughly linear relationship with the percentage of genome encoding BGCs, with some notable outliers showing up as the number of BGCs increases. All genera with above average percentages of the genome dedicated to BGCs are labeled. The average is indicated by AVG. See Supplementary Table 6 for genus abbreviations.



Extended Data Fig. 3. (A) Relationship between intact BGCs and genome quality. In general, genomes with larger N50 values display a higher rate of BGCs predicted to be intact by antiSMASH. [6] However, even genomes with small N50 values contain large intact BGCs. BGCs are predicted as intact if the antiSMASH-annotated boundaries do not stop at a contig edge. (B) Proportion of intact BGCs per GCF in NPDC Actinobacteria genomes. Most NPDC GCFs consist only of intact BGCs, while >99% of NPDC GCFs contain at least one BGC predicted to be intact. BGCs are predicted as intact if the antiSMASH-annotated boundaries do not stop at a contig edge. By using GCFs in place of BGCs for measuring biosynthetic diversity, GCFs containing at least one putative intact BGC can minimize the impact of fragmented BGCs on downstream analyses.

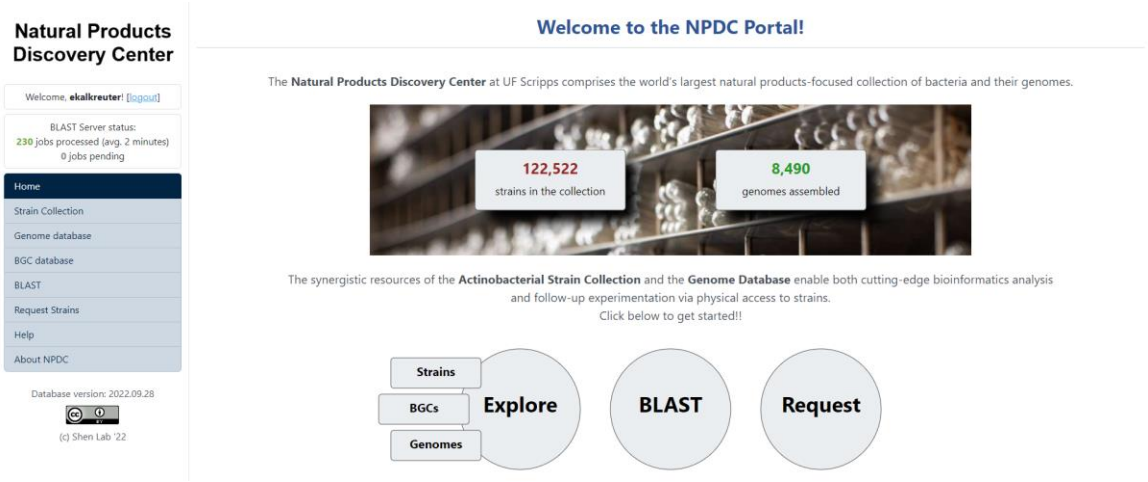
A

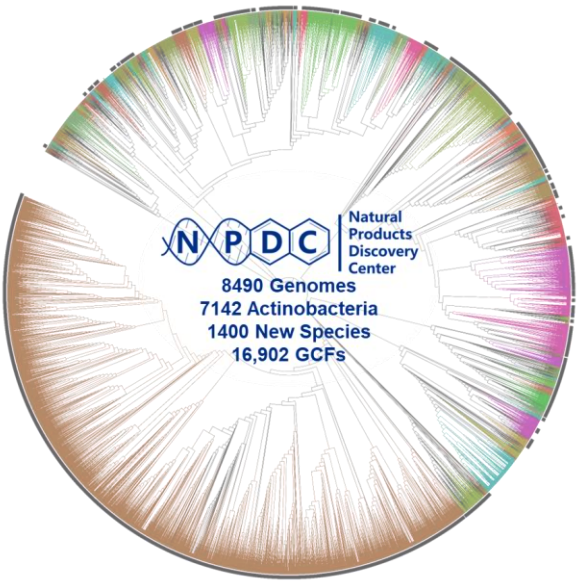


B

Genus	Most Common GCF	Strains	Frequency
ASY	29	23	126.1%
KTS	218	213	102.3%
MCC	53	53	100.0%
KRB	37	37	100.0%
PAC	41	42	97.6%
NCP	40	41	97.6%
STS	48	50	96.0%
NCD	186	194	95.9%
MCM	299	317	94.3%
SPL	24	26	92.3%
MBP	22	24	91.7%
RHD	51	56	91.1%
ACP	29	32	90.6%
AMY	54	62	87.1%
NMU	101	125	80.8%
ART	23	32	71.9%
STR	3776	5386	70.1%
MCB	56	101	55.4%

Extended Data Fig. 4. Distribution of NPDC Actinobacteria GCFs. (A) Distribution of GCFs were plotted based on how many NPDC Actinobacteria genomes they were found in, showing that most GCFs are found in ten or fewer NPDC genomes. (B) In genera with at least 20 strains, the number of instances of the most common GCF in each genus was plotted. In most genera, the most common GCF appears in >90% of genomes in that genus. In some cases, BGCs belonging to the most common GCF appear multiple times within a single genome, leading to percentages >100%.



Extended Data Fig. 5. NPDC Portal home page. From the home page, users can access the strain, BGC, and genome databases, the BLASTP tool, and can request strains. Image pulled March 31, 2023.

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