IMG-ABC: A Knowledge Base To Fuel Discovery of Biosynthetic Gene Clusters and Novel Secondary Metabolites

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ABSTRACT **In the discovery of secondary metabolites, analysis of sequence data is a promising exploration path that remains largely underutilized due to the lack of computational platforms that enable such a systematic approach on a large scale. In this work, we present IMG-ABC (https://img.jgi.doe.gov/abc), an atlas of biosynthetic gene clusters within the Integrated Microbial Genomes (IMG) system, which is aimed at harnessing the power of "big" genomic data for discovering small molecules. IMG-ABC relies on IMG's comprehensive integrated structural and functional genomic data for the analysis of biosynthetic gene clusters (BCs) and associated secondary metabolites (SMs). SMs and BCs serve as the two main classes of objects in IMG-ABC, each with a rich collection of attributes. A unique feature of IMG-ABC is the incorporation of both experimentally validated and computationally predicted BCs in genomes as well as metagenomes, thus identifying BCs in uncultured populations and rare taxa. We demonstrate the strength of IMG-ABC's focused integrated analysis tools in enabling the exploration of microbial secondary metabolism on a global scale, through the discovery of phenazine-producing clusters for the first time in *Alphaproteobacteria*. IMG-ABC strives to fill the long-existent void of resources for computational exploration of the secondary metabolism universe; its underlying scalable framework enables traversal of uncovered phylogenetic and chemical structure space, serving as a doorway to a new era in the discovery of novel molecules.**

IMPORTANCE **IMG-ABC is the largest publicly available database of predicted and experimental biosynthetic gene clusters and the secondary metabolites they produce. The system also includes powerful search and analysis tools that are integrated with IMG's extensive genomic/metagenomic data and analysis tool kits. As new research on biosynthetic gene clusters and secondary metabolites is published and more genomes are sequenced, IMG-ABC will continue to expand, with the goal of becoming an essential component of any bioinformatic exploration of the secondary metabolism world.**

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Secondary metabolites (SMs) are small organic compounds that are not essential to the life of an organism but have, nevertheless, a broad biological activity spectrum. SMs derived from plants have been used for thousands of years in the form of natural extracts due to their pharmacological properties (1), while in the modern era, SMs from plants and microorganisms have been a rich source of therapeutics (2). Certain classes of SMs, such as terpenes, are good candidates for biofuel production (3). SMs serve as important chemical agents of communication between bacteria in complex communities or in symbiotic relationships, such as in plant-microbe interactions (4). In this context, some SMs provide biological control of plant pathogens and thus may have important agricultural applications (5). Discovery of new SMs, therefore, will benefit the development of novel biotechnological applications and provide better understanding of the interactions within complex communities.

Traditionally, microbial SMs have been isolated by screening cultured microbes for the desired pharmacological and/or biological activity (6). This approach has its limitations, since many microorganisms are difficult or impossible to obtain in pure culture. Additionally, the bioactive chemicals may not be produced due to the absence of specific environmental stimuli or may remain undetectable through conventional screening methods (7–9). The rapid growth of genomic data from both isolate organisms and microbial communities (metagenomes) (10), in conjunction with the development of tools for computational identification and classification of biosynthetic gene clusters (BCs) (11, 12), present a new opportunity for the discovery of SMs with novel chemical structures (13). Computationally identified BCs can be cloned or synthetically reconstructed and expressed in heterologous systems, which can then be monitored for the production of potentially novel metabolites. Further, the application of this approach

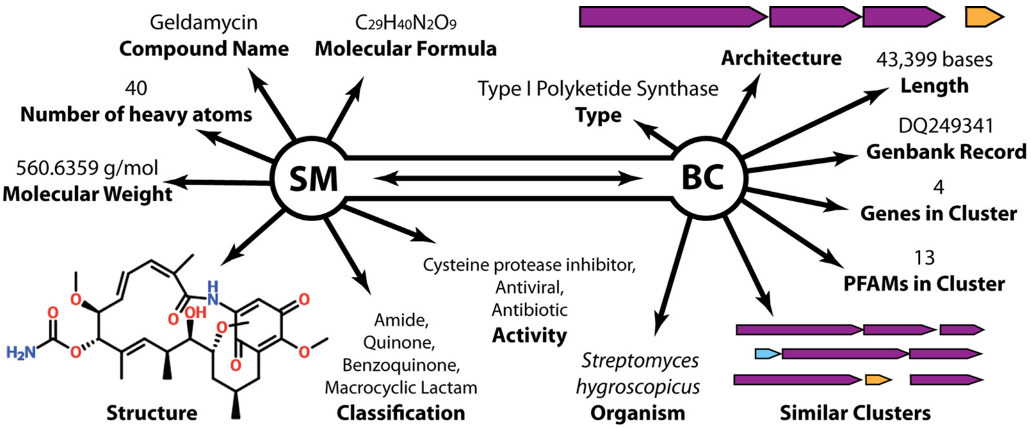


FIG 1 IMG-ABC object structure overview. IMG-ABC contains two main classes of objects with a variety of attributes describing predicted and experimentally studied biosynthetic gene clusters and the secondary metabolites associated with the latter.

to uncultured strains in single cells and metagenomes enables the detection of SM pathways in microbial dark matter (14).

In response to an increasing interest in the identification of novel microbial SMs, a number of databases have been developed in recent years with information on BCs and SMs. StreptomeDB is a database focused on bioactive molecules produced by members of the *Streptomyces* genus (15). DoBISCUIT provides carefully curated annotations of a limited number of BCs (16), while Clustermine360 focuses on polyketide synthase (PKS) and nonribosomal peptide synthetase (NRPS) type clusters and allows for user submissions of clusters (17). ClustScanDB provides detailed information about thiotemplate modular systems (18). Although some of these efforts provide useful high-quality manually curated annotations, they come at the cost of narrow specificity and often have no or limited maintenance. Most of these systems are limited either by specific classes of organisms (e.g., *Streptomyces*) or biosynthetic cluster types (e.g., NRPS/PKS). Additionally, some have relatively few records and do not provide the tools for in-depth sequence analyses, while others are no longer updated.

In order to alleviate some of the above limitations and to pave the way from big data to small molecules, we have developed IMG-ABC, an *a*tlas of *b*iosynthetic gene *c*lusters within the Integrated Microbial Genomes (IMG) system. IMG-ABC is a database of biosynthetic gene clusters and the chemicals they are known to produce and, through its integration with the IMG data management system (10, 19), it provides the following capabilities: (i) access to an exhaustive collection of both predicted and published BCs in over 23,000 public isolate microbial genomes and more than 2,200 metagenomes; (ii) class-agnostic inclusion of all classes and types of BCs and SMs; (iii) integration with structural and functional annotations and metadata from IMG and the Genomes Online Database (GOLD) (20); (iv) enhanced search and analysis capabilities; (v) expert user curation capability; (vi) a track record of continuous maintenance and user support.

As newly sequenced data sets are integrated into IMG, they are automatically processed through IMG's BC prediction pipeline, constantly feeding IMG-ABC with new putative BCs. We expect IMG-ABC to become the primary starting point for the sequence analysis of BCs and to provide the knowledge base needed for the heterologous expression of BCs that produce novel and potentially useful chemical compounds.

RESULTS

Database structure and content. A schematic overview of the IMG-ABC object structure with associated attributes for BCs and SMs is illustrated in Fig. 1. Experimentally described BCs are linked with the SMs they produce, when known. The result of this computationally intensive effort was the creation of a large database of predicted and experimentally verified BCs. IMG-ABC contains more than 750,000 BCs in publicly available records, most of them from bacterial isolate genomes (Table 1) from 57 phyla (Fig. 2A), reflecting the diversity of available genomic sequences in the IMG database. A subset of BCs from isolate genomes can be assigned to one or more BC enzymatic types based on the presence of signature core enzymes (Fig. 2B). The most common enzymatic activity found in the predicted clusters is that of nonribosomal peptide synthetase (21), followed by type I PKS (22). Integration with IMG allows access to metadata annotations from GOLD (20), enabling the connection of information on secondary metabolism and habitat (Fig. 2C). Since *Streptomyces* are an excellent source of natural products and have been studied in this respect more than other genera, more than 60% of SMs with a known chemical structure in IMG-ABC have been isolated from *Actinobacteria* (Fig. 2D).

TABLE 1 IMG-ABC content (BCs) within various domains of life and DNA fragments[a]

Domain or DNA fragment (no. of public samples with BCs)	No. of BCs
Bacteria (23,423)	441,881
Metagenomes (2,230)	260,462
Eukaryota (186)	42,072
Archaea (498)	4196
Viruses (920)	1223
Bacterial genome fragments (1,116)	1391
Bacterial plasmids (197)	271
Eukaryotic genome fragments (139)	164
Eukaryotic plasmids (7)	7
Other plasmids (1)	1
Archaeal genome fragments (2)	1
Total	758,469

[a] Distribution of BCs within various domains of life, genome fragments, and plasmids in IMG and IMG/M (as of 27 January 2015).

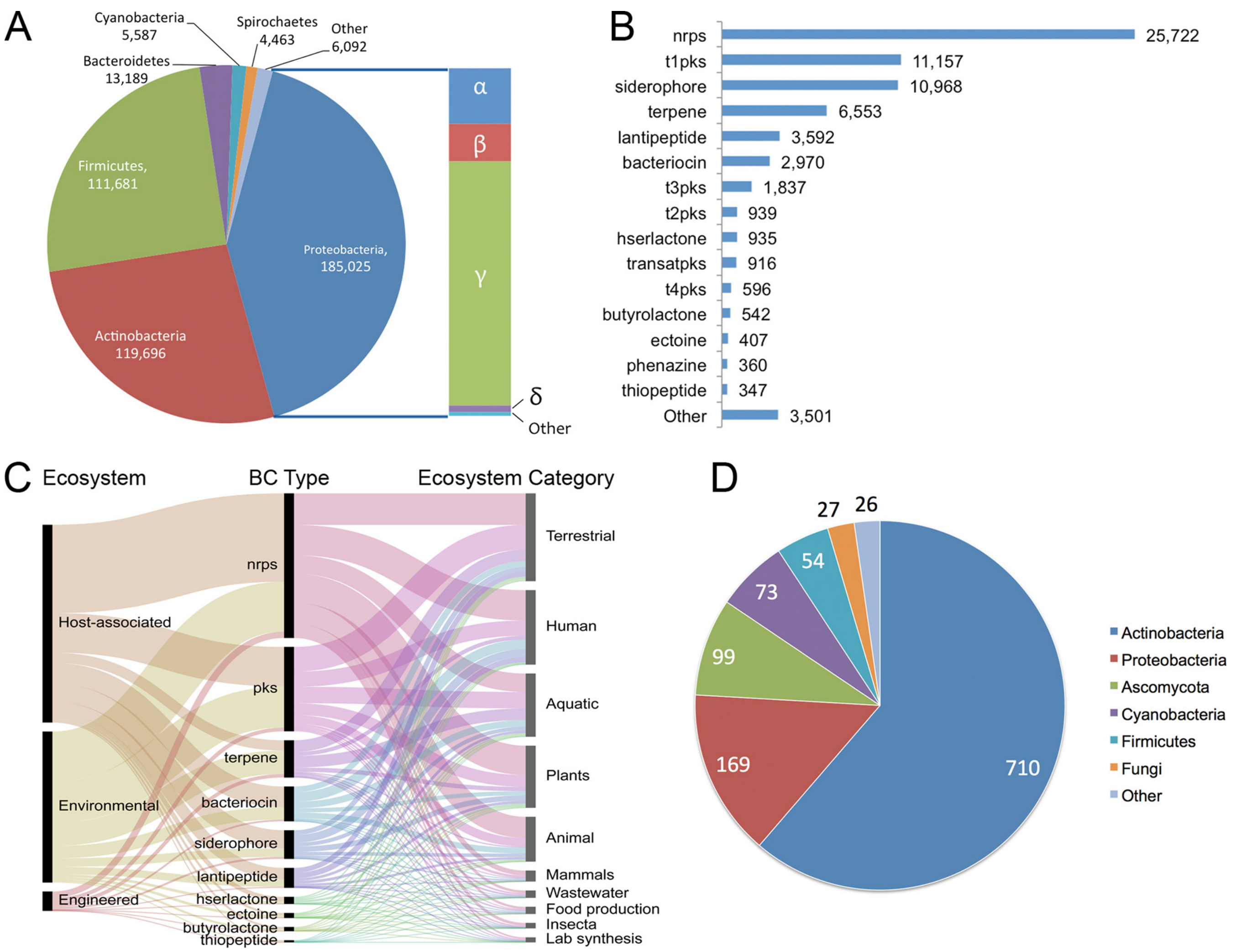
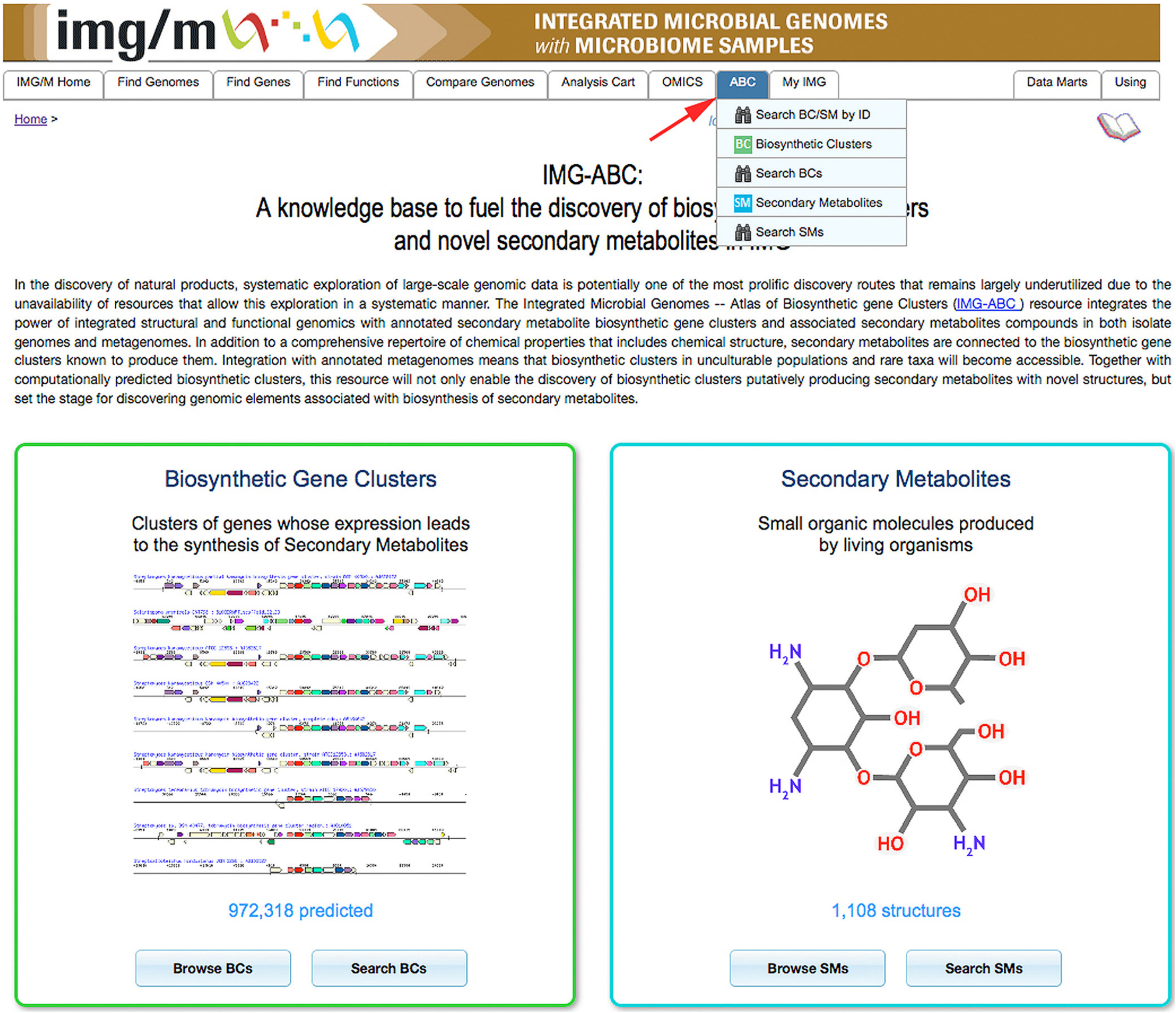


FIG 2 Data content of IMG-ABC. (A) Biosynthetic clusters in isolate bacteria come from a variety of phyla, the most predominant being *Gammaproteobacteria*. (B) Distribution of the top classes of enzymatic activities (BC types) found in bacteria and genome fragments available in IMG-ABC. Abbreviations: nrps, nonribosomal peptide synthetase; t1pks, type I PKS; t3pks, type III PKS; t2pks, type II PKS; hserlactone, homoserine lactone; transatpks, *trans*-AT PKS. (C) Ecosystem sources for the 10 most common BC enzymatic types. (D) Sources of experimentally verified bacterial secondary metabolites, summarized at the phylum level.

Accessing the IMG-ABC system. IMG-ABC can be accessed directly (https://img.jgi.doe.gov/abc) (Fig. 3) or through its seamless integration with the user interface structure of the Joint Genome Institute's (JGI) IMG system (https://img.jgi.doe.gov/) via the "ABC" tab. The synergies gained add an extraordinary value to the IMG-ABC database, because the user has immediate access not only to both computationally predicted and experimentally validated BCs and associated SMs but also to a vast array of integrated functionally and phylogenetically annotated genomic/metagenomic data, gene expression data, and functional genomics data. Users also have access to search-based and statistics-based entry points into both BC and SM objects within IMG. Most analysis tools in IMG are accessible to workflows employing an SM or a BC as a starting point. An important added benefit stemming from IMG-ABC's integration with IMG is that it will help expose secondary metabolism to the 10,000+ registered IMG users, some of whom may have never before considered studying BCs in their favorite organisms, and will empower them to quickly and easily ask questions using a familiar interface.

Browsing the BC database. The entry page to the BC statistics section of IMG-ABC displays the summary statistics for the BC data available in the system (Fig. 4A). Additional tabs allow the user to browse BCs by domain, phylum, BC type, SM type, cluster length, gene count, and functional classifications (*viz.* Enzyme Commission [EC] numbers [23] and Pfams [24]) assigned to genes within them. The summary data for BCs found under each tab are further broken down by experimental versus predicted and also by whether they are found in isolate genomes or in metagenomes. Narrowing results to specific categories is click-enabled for the user to arrive at a limited number of BCs of interest. Browsing BCs by their primary enzymatic mechanism ("BC type") helps narrow search results to relevant BC types (Fig. 4B). Similarly, since specific Pfams and EC numbers can be directly related to secondary metabolism, browsing BCs by Pfams and EC numbers (Fig. 4C) is a useful feature that enables the analysis of gene clusters, but this feature is not available in any existing application. Once users obtain a listing of BCs in a tabular form by using any of the browsing methods mentioned above, they have the option to add genes of se-



IMG-ABC:
A knowledge base to fuel the discovery of bios... ...rs
and novel secondary metabolites ...

In the discovery of natural products, systematic exploration of large-scale genomic data is potentially one of the most prolific discovery routes that remains largely underutilized due to the unavailability of resources that allow this exploration in a systematic manner. The Integrated Microbial Genomes -- Atlas of Biosynthetic gene Clusters (IMG-ABC) resource integrates the power of integrated structural and functional genomics with annotated secondary metabolite biosynthetic gene clusters and associated secondary metabolites compounds in both isolate genomes and metagenomes. In addition to a comprehensive repertoire of chemical properties that includes chemical structure, secondary metabolites are connected to the biosynthetic gene clusters known to produce them. Integration with annotated metagenomes means that biosynthetic clusters in unculturable populations and rare taxa will become accessible. Together with computationally predicted biosynthetic clusters, this resource will not only enable the discovery of biosynthetic clusters putatively producing secondary metabolites with novel structures, but set the stage for discovering genomic elements associated with biosynthesis of secondary metabolites.

Biosynthetic Gene Clusters

Clusters of genes whose expression leads to the synthesis of Secondary Metabolites

972,318 predicted

Secondary Metabolites

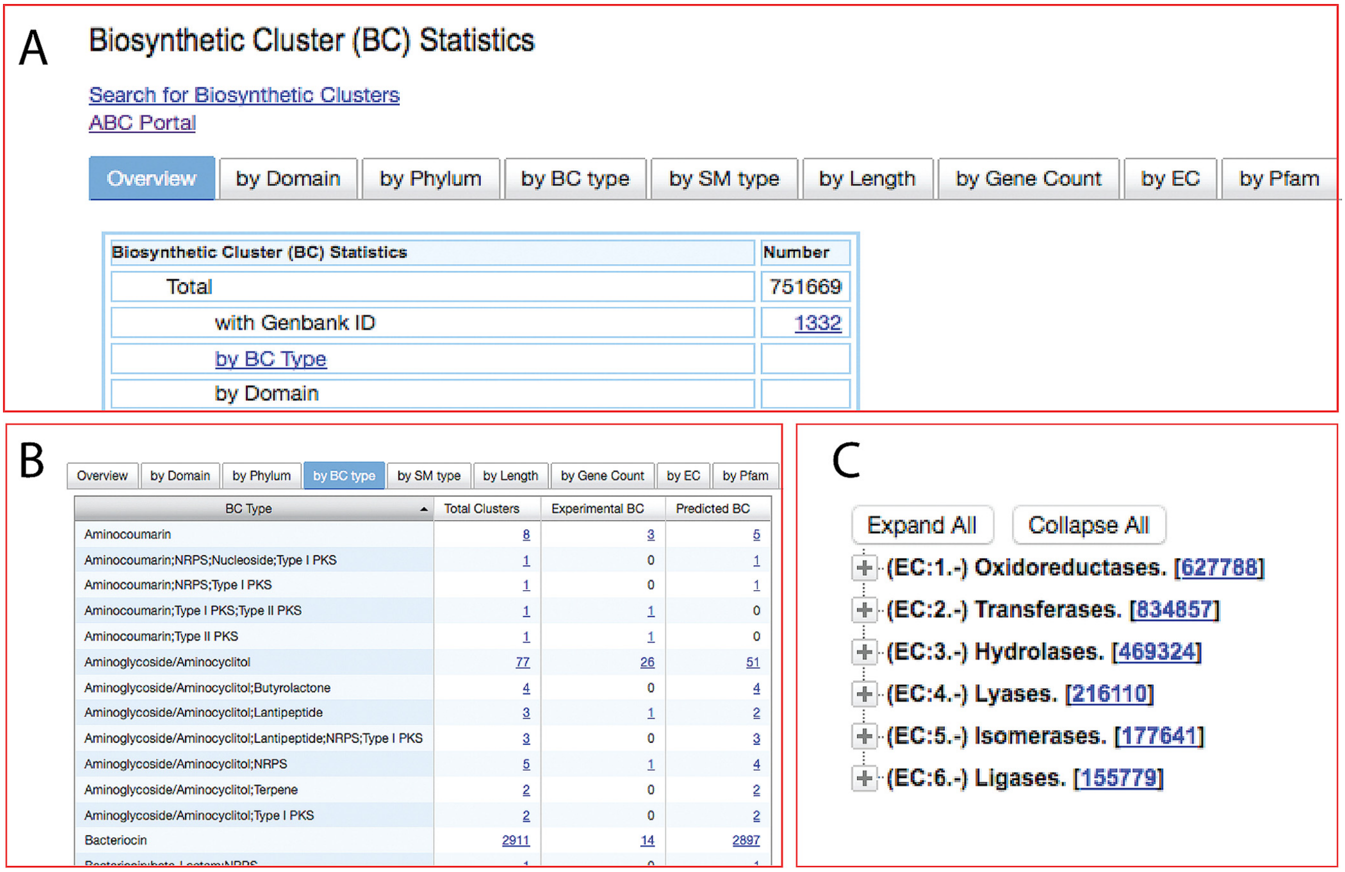
Small organic molecules produced by living organisms

1,108 structures

FIG 3 Entry page to the IMG-ABC system. IMG-ABC is accessible either directly at https://img.jgi.doe.gov/abc or through the "ABC" tab in IMG (red arrow). Through these two avenues, the user has access to functions for the analysis and search of SMs and BCs.

lected BCs to IMG's "Gene Cart" for further analysis. There is also the capability to directly add the scaffolds where the selected BCs are found to the "Scaffold Cart," which provides access to IMG's extensive arsenal of tools for statistical and phylogenetic analyses.

BC detail interface. Once users obtain a BC of interest, they can unlock a new set of BC-specific interfaces. The entry tab displays more information pertaining to the BC (Fig. 5A). Additionally, users can export the BC-related sequence in fasta format (for nucleotides or amino acids) or GenBank format. The "Genes in Cluster" tab lists all genes in the BC along with functional annotations and the bidirectional best hit for the gene and genome (Fig. 5C). From this tab, the user can add a subset or all genes to the Gene Cart for further analysis or storage. The "Cluster Neighborhood" tab provides access to an interactive graphical representation of the BC architecture along with its flanking regions. The BC boundary is denoted by a red dashed box, and each gene is colored according to its assigned COG. Clicking on any gene will result in displaying the neighborhoods of the bidirectional best hits for that gene, ordered by descending bit score (Fig. 5D). This function is a powerful way to evaluate potentially related loci and quickly highlight variant cluster architectures or unusual enzymatic activities, thus leading to the discovery of novel secondary metabolites. If the BC viewed is an experimentally validated one, the "Secondary Metabolite" tab will list the SMs that are produced by the BC whenever the chemical structure is available. The last three tabs list the metabolic pathways (KEGG, MetaCyc, IMG pathways) (Fig. 5B) in which the genes participate. KEGG and MetaCyc pathway detail pages show both genes in the selected BC and genes not in this BC but in the same genome. This provides users with the capability to understand the role of a BC inside a genome and to further investigate related genes functioning in the same pathway.



Biosynthetic Cluster (BC) Statistics	Number
Total	751669
with Genbank ID	1332
by BC Type	
by Domain	

BC Type	Total Clusters	Experimental BC	Predicted BC
Aminocoumarin	8	3	5
Aminocoumarin;NRPS;Nucleoside;Type I PKS	1	0	1
Aminocoumarin;NRPS;Type I PKS	1	0	1
Aminocoumarin;Type I PKS;Type II PKS	1	1	0
Aminocoumarin;Type II PKS	1	1	0
Aminoglycoside/Aminocyclitol	77	26	51
Aminoglycoside/Aminocyclitol;Butyrolactone	4	0	4
Aminoglycoside/Aminocyclitol;Lantipeptide	3	1	2
Aminoglycoside/Aminocyclitol;Lantipeptide;NRPS;Type I PKS	3	0	3
Aminoglycoside/Aminocyclitol;NRPS	5	1	4
Aminoglycoside/Aminocyclitol;Terpene	2	0	2
Aminoglycoside/Aminocyclitol;Type I PKS	2	0	2
Bacteriocin	2911	14	2897
Bacteriocin;beta-Lactam;NRPS	1	0	1

- (EC:1.-) Oxidoreductases. [627788]
- (EC:2.-) Transferases. [834857]
- (EC:3.-) Hydrolases. [469324]
- (EC:4.-) Lyases. [216110]
- (EC:5.-) Isomerases. [177641]
- (EC:6.-) Ligases. [155779]

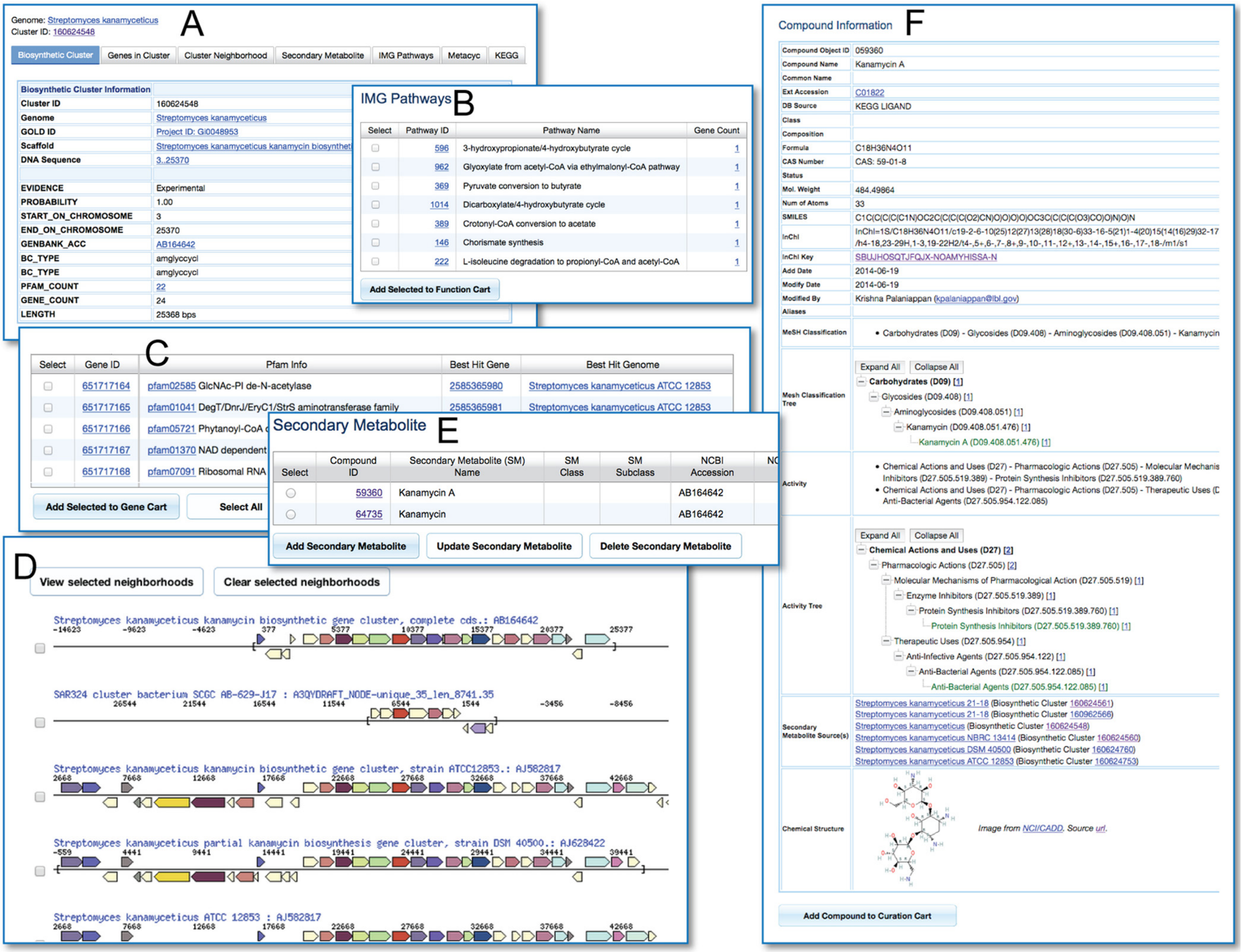
FIG 4 BC summary section. (A) The BC statistics section can be navigated via the multiple-tab interface. (B) Listing of the number of BCs associated with specific combinations of BC types. (C) An expandable and interactive hierarchical tree of Enzyme Commission (EC) enzyme classifications.

Browsing the SM database. Similarly to BCs, the landing page to the SM Statistics section provides an overview of the database content. Through the multitabbed layout, SMs are displayed by type of chemical structure (per MeSH [25]), a tabular listing, pharmacological activity, and phylum. All compounds are integrated with structural and activity classifications available via MeSH on the PubChem website (26) and can be visualized as a clickable tree interface.

SM detail interface. The page for each SM contains extensive information and links to external databases associated with the compound of interest. Additionally, it includes lists and links to all BCs within IMG-ABC that are connected with that compound (Fig. 5E). Besides the essential chemical descriptors (e.g., molecular weight, chemical formula, etc.), users have direct access to SMILES and InChI identifiers that can be linked to metabolites present in available genome-scale metabolic models (e.g., *Streptomyces* models that use flux balance to predict antibiotic production [27]), which can aid in the reconstruction of genome-scale models for SM production in less-studied microorganisms. The compound detail page also provides clickable interfaces for both structural classifications and activity hierarchical trees. Last, this page displays the two-dimensional chemical structure of the compound (Fig. 5F).

Searching biosynthetic clusters and secondary metabolites by annotation. The IMG-ABC database contains a large number of experimentally validated and predicted BCs, with the former group connected to the SMs that they are known to produce. To enable users to efficiently narrow their search based on their specific interests, two search interfaces can be implemented (Fig. 6). Both SMs and BCs can be searched by a combination of chemical names, SM chemical classification, SM activity, number of atoms, molecular weight, and chemical formula, while the search space can be limited to specific target clades through a phylogenetic tree-based menu. Additionally, BCs are searchable by genomic features such as the number of genes, length of the BC, probability of the prediction, and type of enzymatic mechanism in the BC assigned by the AntiSMASH tool (12). Since there can be multiple combinations of BC types assigned to a cluster, we also provide users with the option to perform exact or inexact (and/or) searches for BCs with hybrid types. Another useful option is the ability to limit the results based on the presence of a single Pfam or combinations thereof, which enables "fishing" for enzymatic activities that may not be included in the AntiSMASH classification routine and therefore can aid in the discovery of new enzymatic mechanisms and/or novel chemical structures. The results are displayed in a tabular form and, in the case of BC searches, users can add selected BCs to the "Scaffold Cart" for further analysis (Fig. 6A).

Searching IMG-ABC based on a chemical structure. The ability to use a chemical structure to query a genomic database is one of the new and exciting features introduced by IMG-ABC (Fig. 6B). A known SMILES descriptor (the user's own or one retrieved through an external interface, such as NCBI PubChem's


A
Biosynthetic Cluster
Genes in Cluster
Cluster Neighborhood
Secondary Metabolite
IMG Pathways
Metacyc
KEGG
B
IMG Pathways
Add Selected to Function Cart
C
Add Selected to Gene Cart
Select All
E
Secondary Metabolite
Add Secondary Metabolite
Update Secondary Metabolite
Delete Secondary Metabolite
D
View selected neighborhoods
Clear selected neighborhoods
Streptomyces kanamyceticus kanamycin biosynthetic gene cluster, complete cds.: AB164642
-14623 -9623 -4623 377 5377 10377 15377 20377 25377
SAR324 cluster bacterium SCGC AB-629-J17 : A3QYDRAFT_NODE-unique_35_len_8741.35
26544 21544 16544 11544 6544 1544 -3456 -8456
Streptomyces kanamyceticus kanamycin biosynthetic gene cluster, strain ATCC12853.: AJ582817
2668 7668 12668 17668 22668 27668 32668 37668 42668
Streptomyces kanamyceticus partial kanamycin biosynthesis gene cluster, strain DSM 40500.: AJ628422
-559 4441 9441 14441 19441 24441 29441 34441 39441
Streptomyces kanamyceticus ATCC 12853 : AJ582817
2668 7668 12668 17668 22668 27668 32668 37668 42668
F
Compound Information
Expand All
Collapse All
Add Compound to Curation Cart

Genome: Streptomyces kanamyceticus
Cluster ID: 160624548

Biosynthetic Cluster Information	
Cluster ID	160624548
Genome	Streptomyces kanamyceticus
GOLD ID	Project ID: Gi0048953
Scaffold	Streptomyces kanamyceticus kanamycin biosynthet
DNA Sequence	3..25370
EVIDENCE	Experimental
PROBABILITY	1.00
START_ON_CHROMOSOME	3
END_ON_CHROMOSOME	25370
GENBANK_ACC	AB164642
BC_TYPE	amglyccycl
BC_TYPE	amglyccycl
PFAM_COUNT	22
GENE_COUNT	24
LENGTH	25368 bps

Select	Pathway ID	Pathway Name	Gene Count
□	596	3-hydroxypropionate/4-hydroxybutyrate cycle	1
□	962	Glyoxylate from acetyl-CoA via ethylmalonyl-CoA pathway	1
□	369	Pyruvate conversion to butyrate	1
□	1014	Dicarboxylate/4-hydroxybutyrate cycle	1
□	389	Crotonyl-CoA conversion to acetate	1
□	146	Chorismate synthesis	1
□	222	L-isoleucine degradation to propionyl-CoA and acetyl-CoA	1

Select	Gene ID	Pfam Info	Best Hit Gene	Best Hit Genome
□	651717164	pfam02585 GlcNAc-PI de-N-acetylase	2585365980	Streptomyces kanamyceticus ATCC 12853
□	651717165	pfam01041 DegT/DnrJ/EryC1/StrS aminotransferase family	2585365981	Streptomyces kanamyceticus ATCC 12853
□	651717166	pfam05721 Phytanoyl-CoA d		
□	651717167	pfam01370 NAD dependent		
□	651717168	pfam07091 Ribosomal RNA		

Select	Compound ID	Secondary Metabolite (SM) Name	SM Class	SM Subclass	NCBI Accession	NC
○	59360	Kanamycin A			AB164642	
○	64735	Kanamycin			AB164642	

Compound Object ID	059360
Compound Name	Kanamycin A
Common Name	
Ext Accession	C01822
DB Source	KEGG LIGAND
Class	
Composition	
Formula	C18H36N4O11
CAS Number	CAS: 59-01-8
Status	
Mol. Weight	484.49864
Num of Atoms	33
SMILES	C1C(C(C(C(C1N)OC2C(C(C(C(O2)CN)O)O)O)O)OC3C(C(C(C(O3)CO)O)N)O)N
InChI	InChI=1S/C18H36N4O11/c19-2-6-10(25)12(27)13(28)18(30-6)33-16-5(21)1-4(20)15(14(16)29)32-17 /h4-18,23-29H,1-3,19-22H2/t4-,5+,6-,7-,8+,9-,10-,11-,12+,13-,14-,15+,16-,17-,18-/m1/s1
InChI Key	SBUJHOSQTJFQJX-NOAMYHISSA-N
Add Date	2014-06-19
Modify Date	2014-06-19
Modified By	Krishna Palaniappan (kpalaniappan@lbl.gov)
Aliases	
MeSH Classification	• Carbohydrates (D09) - Glycosides (D09.408) - Aminoglycosides (D09.408.051) - Kanamycin
Mesh Classification Tree	Carbohydrates (D09) [1] Glycosides (D09.408) [1] Aminoglycosides (D09.408.051) [1] Kanamycin (D09.408.051.476) [1] Kanamycin A (D09.408.051.476) [1]
Activity	• Chemical Actions and Uses (D27) - Pharmacologic Actions (D27.505) - Molecular Mechanis Inhibitors (D27.505.519.389) - Protein Synthesis Inhibitors (D27.505.519.389.760) • Chemical Actions and Uses (D27) - Pharmacologic Actions (D27.505) - Therapeutic Uses (D Anti-Bacterial Agents (D27.505.954.122.085)
Activity Tree	Chemical Actions and Uses (D27) [2] Pharmacologic Actions (D27.505) [2] Molecular Mechanisms of Pharmacological Action (D27.505.519) [1] Enzyme Inhibitors (D27.505.519.389) [1] Protein Synthesis Inhibitors (D27.505.519.389.760) [1] Protein Synthesis Inhibitors (D27.505.519.389.760) [1] Therapeutic Uses (D27.505.954) [1] Anti-Infective Agents (D27.505.954.122) [1] Anti-Bacterial Agents (D27.505.954.122.085) [1] Anti-Bacterial Agents (D27.505.954.122.085) [1]
Secondary Metabolite Source(s)	Streptomyces kanamyceticus 21-18 (Biosynthetic Cluster 160624561) Streptomyces kanamyceticus 21-18 (Biosynthetic Cluster 160962566) Streptomyces kanamyceticus (Biosynthetic Cluster 160624548) Streptomyces kanamyceticus NBRC 13414 (Biosynthetic Cluster 160624560) Streptomyces kanamyceticus DSM 40500 (Biosynthetic Cluster 160624760) Streptomyces kanamyceticus ATCC 12853 (Biosynthetic Cluster 160624753)
Chemical Structure	Image from NCI/CADD. Source url.

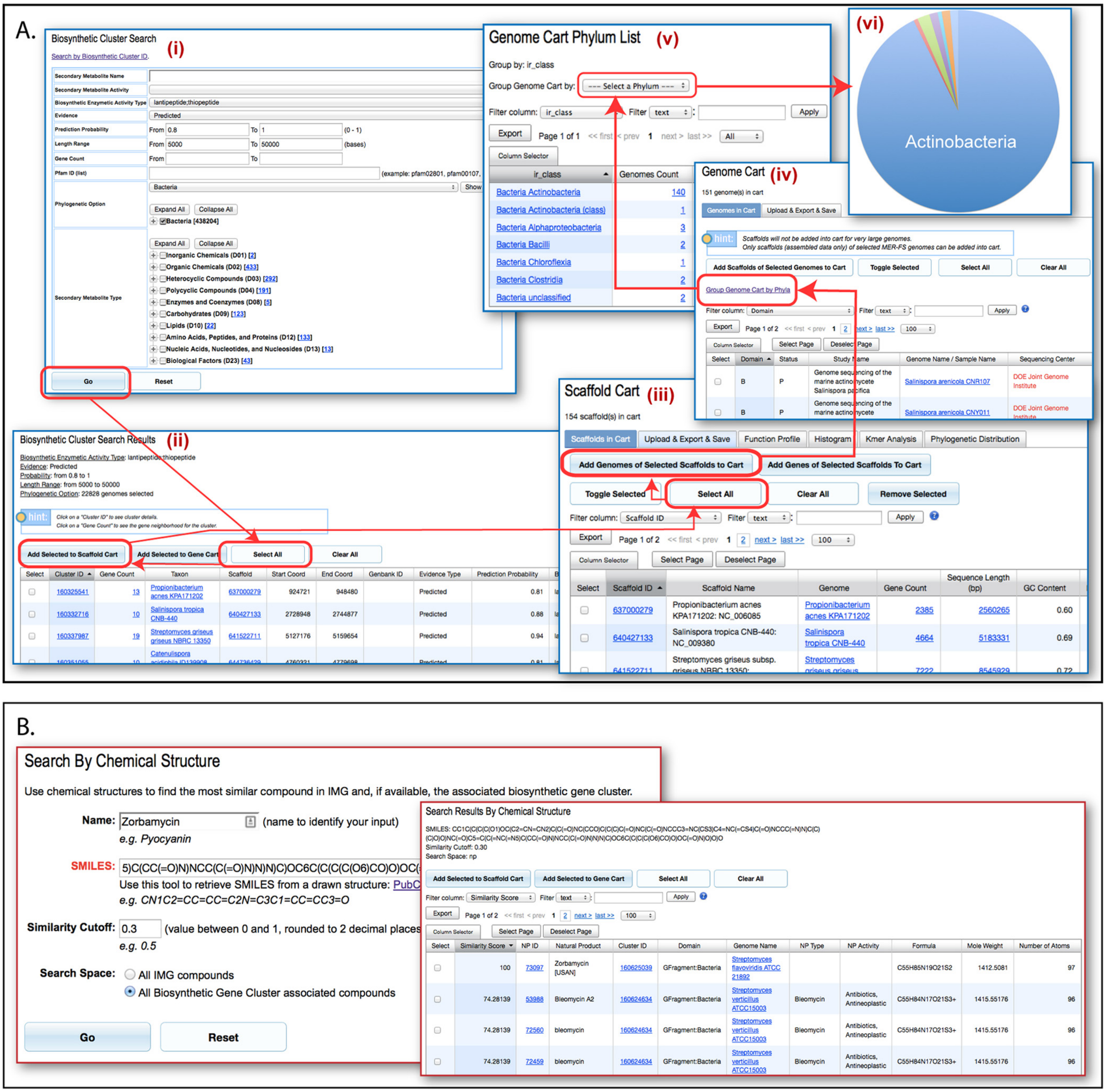
FIG 5 Biosynthetic cluster and secondary metabolite detail interfaces. (A) Interfaces to browse detailed statistics. (B) Pathway relationships. (C) Gene content. (D) Neighborhood views of bidirectional best-hit searches. (E) Secondary metabolite information for experimentally validated BCs. (F) Detailed compound annotations for SMs.

Sketcher) is used to search compounds based on the structural similarity, computed using the Tanimoto similarity score (28, 29) to produce an interactive listing of similar SMs and related BCs, sorted by the similarity score. This search is powered through an implementation of ChemmineR functions (29) and facilitated by the precomputed atom pair descriptors for all compounds in the IMG-ABC database. SMILES strings entered by users are converted to a structure-data file (SDF) format and then to atom pair descriptors, which are used to search either all secondary metabolites or all IMG compounds, depending on the user's input.

Case study: analysis of the distribution of putative phenazine-encoding BCs. A case study illustrating the power of IMG-ABC in exploration and discovery of novel BCs operating on big biological data is presented below. A global survey of BCs containing at least six of the seven core genes essential for phenazine biosynthesis (30) was conducted against the full complement of 25,000+ isolate genomes within IMG-ABC. The resulting set of nearly 1,000 hits was filtered to retain putative complete BCs present on longer scaffolds, which were subsequently narrowed to 26 phenazine and phenazine-hybrid BCs with unique pathway architectures (Fig. 7A). In addition, to establish gene clusters for phenazine biosynthesis, this analysis identified several unique and potentially novel phenazine pathway architectures in *Gammaproteobacteria*, *Betaproteobacteria*, and *Actinobacteria*, opening doors for the discovery of novel enzymatic activities and/or phenazine derivatives. Additionally, pathways for phenazine biosynthesis were detected for the first time in two alphaproteobacterial genomes: the root-nodulating *Rhizobium leguminosarum* bv. *trifolii* and the photosynthetic *Phaeospirillum molischianum*.

The discovery of a phenazine cluster in a *Rhizobium* species is particularly exciting. The presence of three genes of unknown function and the novel architecture of the cluster (Fig. 7A) suggest that novel enzymatic activities may be involved, and therefore the product may be a phenazine derivative with a novel chemical structure.

Additionally, it appears that this cluster was acquired through horizontal gene transfer. First, the cluster is flanked by transposases (Fig. 7B; see also Table S1 in the supplemental material). A BLASTp search was performed with the proteins in this locus (in



Biosynthetic Cluster Search

Search by Biosynthetic Cluster ID.

Secondary Metabolite Name	
Secondary Metabolite Activity	
Biosynthetic Enzymetic Activity Type	lantipeptide;thiopeptide
Evidence	Predicted
Prediction Probability	From 0.8 To 1 (0 - 1)
Length Range	From 5000 To 50000 (bases)
Gene Count	From To
Pfam ID (list)	(example: pfam02801, pfam00107,
Phylogenetic Option	Bacteria Show Expand All Collapse All ☑Bacteria [438204]
Secondary Metabolite Type	Expand All Collapse All □Inorganic Chemicals (D01) [2] □Organic Chemicals (D02) [433] □Heterocyclic Compounds (D03) [292] □Polycyclic Compounds (D04) [191] □Enzymes and Coenzymes (D08) [5] □Carbohydrates (D09) [123] □Lipids (D10) [22] □Amino Acids, Peptides, and Proteins (D12) [133] □Nucleic Acids, Nucleotides, and Nucleosides (D13) [13] □Biological Factors (D23) [43]

Go Reset

Biosynthetic Cluster Search Results

Biosynthetic Enzymetic Activity Type: lantipeptide;thiopeptide
Evidence: Predicted
Probability: from 0.8 to 1
Length Range: from 5000 to 50000
Phylogenetic Option: 22828 genomes selected

hint: *Click on a "Cluster ID" to see cluster details.*
Click on a "Gene Count" to see the gene neighborhood for the cluster.

Add Selected to Scaffold Cart | Add Selected to Gene Cart | Select All | Clear All

Select	Cluster ID	Gene Count	Taxon	Scaffold	Start Coord	End Coord	Genbank ID	Evidence Type	Prediction Probability
□	160325541	13	Propionibacterium acnes KPA171202	637000279	924721	948480		Predicted	0.81
□	160332716	10	Salinispora tropica CNB-440	640427133	2728948	2744877		Predicted	0.88
□	160337987	19	Streptomyces griseus griseus NBRC 13350	641522711	5127176	5159654		Predicted	0.94
□	160351055	10	Catenulispora acidiphila ID139908	644736429	4760321	4779698		Predicted	0.81

Scaffold Cart

154 scaffold(s) in cart

Scaffolds in Cart | Upload & Export & Save | Function Profile | Histogram | Kmer Analysis | Phylogenetic Distribution

Add Genomes of Selected Scaffolds to Cart | Add Genes of Selected Scaffolds To Cart

Toggle Selected | Select All | Clear All | Remove Selected

Filter column: Scaffold ID Filter text : Apply

Export Page 1 of 2 << first < prev 1 2 next > last >> 100

Column Selector | Select Page | Deselect Page

Select	Scaffold ID	Scaffold Name	Genome	Gene Count	Sequence Length (bp)	GC Content
□	637000279	Propionibacterium acnes KPA171202: NC_006085	Propionibacterium acnes KPA171202	2385	2560265	0.60
□	640427133	Salinispora tropica CNB-440: NC_009380	Salinispora tropica CNB-440	4664	5183331	0.69
□	641522711	Streptomyces griseus subsp. griseus NBRC 13350:	Streptomyces griseus griseus	7222	8545929	0.72

Genome Cart

151 genome(s) in cart

Genomes in Cart | Upload & Export & Save

hint: *Scaffolds will not be added into cart for very large genomes.*
Only scaffolds (assembled data only) of selected MER-FS genomes can be added into cart.

Add Scaffolds of Selected Genomes to Cart | Toggle Selected | Select All | Clear All

Group Genome Cart by Phyla

Filter column: Domain Filter text : Apply

Export Page 1 of 2 << first < prev 1 2 next > last >> 100

Column Selector | Select Page | Deselect Page

Select	Domain	Status	Study Name	Genome Name / Sample Name	Sequencing Center
□	B	P	Genome sequencing of the marine actinomycete Salinispora pacifica	Salinispora arenicola CNR107	DOE Joint Genome Institute
□	B	P	Genome sequencing of the marine actinomycete	Salinispora arenicola CNY011	DOE Joint Genome Institute

Genome Cart Phylum List

Group by: ir_class

Group Genome Cart by: --- Select a Phylum ---

Filter column: ir_class Filter text : Apply

Export Page 1 of 1 << first < prev 1 next > last >> All

Column Selector

ir_class	Genomes Count
Bacteria Actinobacteria	140
Bacteria Actinobacteria (class)	1
Bacteria Alphaproteobacteria	3
Bacteria Bacilli	2
Bacteria Chloroflexia	1
Bacteria Clostridia	2
Bacteria unclassified	2

Search By Chemical Structure

Use chemical structures to find the most similar compound in IMG and, if available, the associated biosynthetic gene cluster.

Name: Zorbamycin (name to identify your input)
e.g. Pyocyanin

SMILES: 5)C(CC(=O)N)NCC(C(=O)N)N)N)C)OC6C(C(C(C(O6)CO)O)OC(
Use this tool to retrieve SMILES from a drawn structure: PubC
e.g. CN1C2=CC=CC=C2N=C3C1=CC=CC3=O

Similarity Cutoff: 0.3 (value between 0 and 1, rounded to 2 decimal places
e.g. 0.5

Search Space: ○ All IMG compounds
● All Biosynthetic Gene Cluster associated compounds

Go Reset

Search Results By Chemical Structure

SMILES: CC1C(C(C(C(O1)OC(C2=CN=CN2)C(C(=O)NC(CCO)C(C(C)C(=O)NC(C(=O)NCCC3=NC(CS3)C4=NC(=CS4)C(=O)NCCC(=N)N)C(C)(C)O)O)NC(=O)C5=C(C(=NC(=N5)C(CC(=O)N)NCC(C(=O)N)N)N)C)OC6C(C(C(C(O6)CO)O)OC(=O)N)O)O)O
Similarity Cutoff: 0.30
Search Space: np

Add Selected to Scaffold Cart | Add Selected to Gene Cart | Select All | Clear All

Filter column: Similarity Score Filter text : Apply

Export Page 1 of 2 << first < prev 1 2 next > last >> 100

Column Selector | Select Page | Deselect Page

Select	Similarity Score	NP ID	Natural Product	Cluster ID	Domain	Genome Name	NP Type	NP Activity	Formula	Mole Weight	Number of Atoms
□	100	73097	Zorbamycin [USAN]	160625039	GFragment:Bacteria	Streptomyces flavoviridis ATCC 21892			C55H85N19O21S2	1412.5081	97
□	74.28139	53988	Bleomycin A2	160624634	GFragment:Bacteria	Streptomyces verticillus ATCC15003	Bleomycin	Antibiotics, Antineoplastic	C55H84N17O21S3+	1415.55176	96
□	74.28139	72560	bleomycin	160624634	GFragment:Bacteria	Streptomyces verticillus ATCC15003	Bleomycin	Antibiotics, Antineoplastic	C55H84N17O21S3+	1415.55176	96
□	74.28139	72459	bleomycin	160624634	GFragment:Bacteria	Streptomyces verticillus ATCC15003	Bleomycin	Antibiotics, Antineoplastic	C55H84N17O21S3+	1415.55176	96

FIG 6 Search interfaces and workflows. (A) Use of the search functions of IMG-ABC in combination with IMG's analysis tools to investigate the distribution of BCs of specific classes. A search for a BC with the desired attributes is performed through the BC search interface (i) results and yields a list of records (ii), which can be added first to IMG's Scaffold Cart (iii) and then to the Genome Cart (iv). From the organisms harboring BCs, matching the initial search can be grouped into different phylogenetic classes (v). These results can be exported for further analysis and visualization (vi). (B) An example of a search using a chemical structure as a query. The chemical structure descriptor string for zorbamycin was used to search IMG-ABC for secondary metabolites that matched the query with a similarity score greater than 0.3. This yielded a list of 118 compounds sorted by descending similarity score whose associated BCs could be analyzed using the Gene Cart and the Scaffold Cart.

the cluster and flanking) against all genomes in IMG. Although all of the flanking proteins (12 upstream and 7 downstream) had their best hit against other alphaproteobacterial proteins, 8 out of the 11 proteins in the cluster were most similar to proteins from the *Actinobacteria* phylum, while 2 were most similar to gammaproteobacterial proteins (Fig. 7B; see also Table S1). The 11th protein was a small hypothetical protein with no hits against the IMG database. The fact that there are 32 genomes (with "permanent draft" or "finished" status) of closely related *Rhizobium leguminosarum* strains in the IMG database and none of them appeared as a top hit in the BLASTp search indicate that the acquisition of these genes must have been recent.

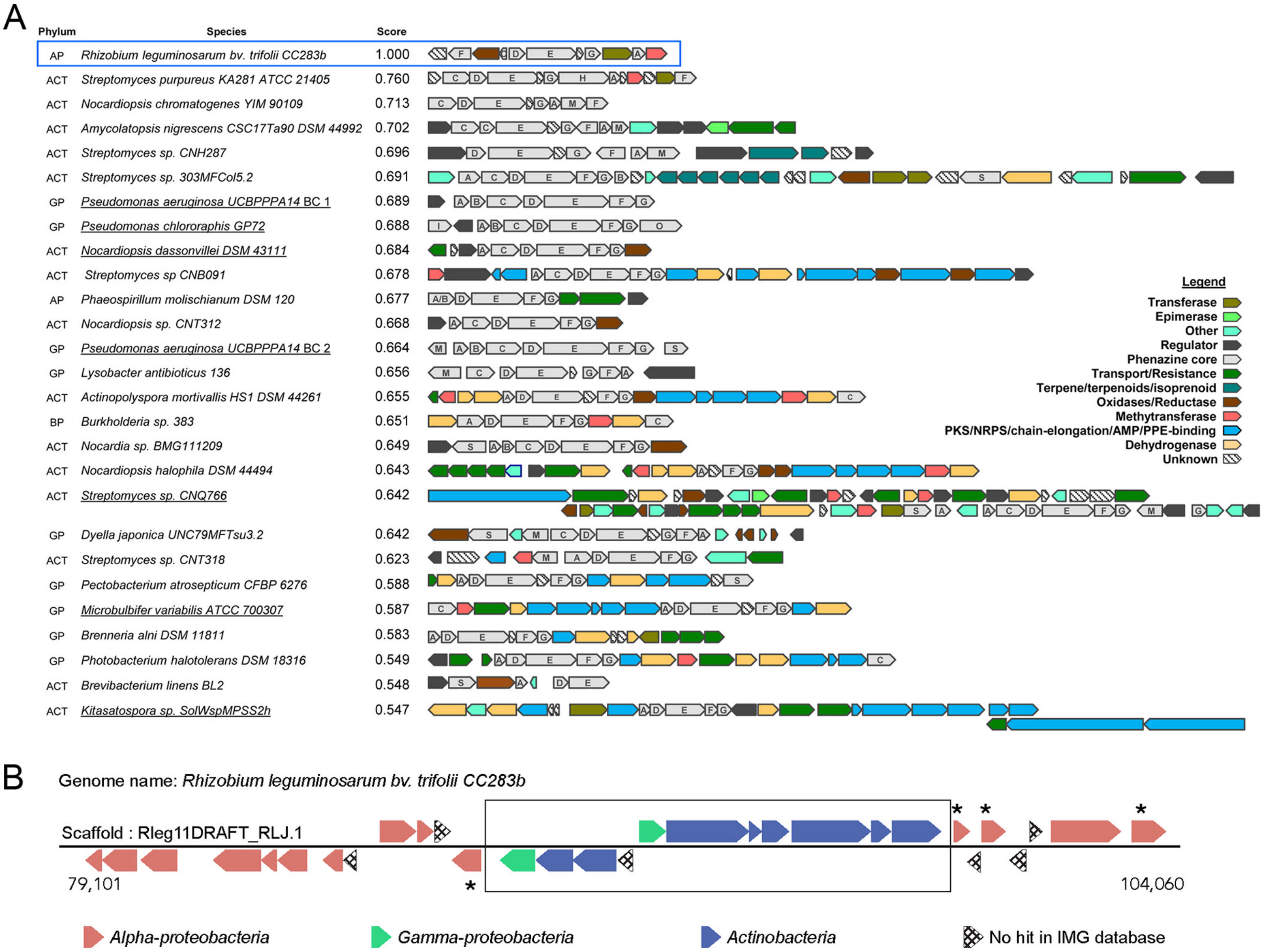

A
Phylum
Species
Score
AP Rhizobium leguminosarum bv. trifolii CC283b 1.000
ACT Streptomyces purpureus KA281 ATCC 21405 0.760
ACT Nocardiopsis chromatogenes YIM 90109 0.713
ACT Amycolatopsis nigrescens CSC17Ta90 DSM 44992 0.702
ACT Streptomyces sp. CNH287 0.696
ACT Streptomyces sp. 303MFCol5.2 0.691
GP Pseudomonas aeruginosa UCBPPPA14 BC 1 0.689
GP Pseudomonas chlororaphis GP72 0.688
ACT Nocardiopsis dassonvillei DSM 43111 0.684
ACT Streptomyces sp CNB091 0.678
AP Phaeospirillum molischianum DSM 120 0.677
ACT Nocardiopsis sp. CNT312 0.668
GP Pseudomonas aeruginosa UCBPPPA14 BC 2 0.664
GP Lysobacter antibioticus 136 0.656
ACT Actinopolyspora mortivallis HS1 DSM 44261 0.655
BP Burkholderia sp. 383 0.651
ACT Nocardia sp. BMG111209 0.649
ACT Nocardiopsis halophila DSM 44494 0.643
ACT Streptomyces sp. CNQ766 0.642
GP Dyella japonica UNC79MFTsu3.2 0.642
ACT Streptomyces sp. CNT318 0.623
GP Pectobacterium atrosepticum CFBP 6276 0.588
GP Microbulbifer variabilis ATCC 700307 0.587
GP Brenneria alni DSM 11811 0.583
GP Photobacterium halotolerans DSM 18316 0.549
ACT Brevibacterium linens BL2 0.548
ACT Kitasatospora sp. SolWspMPSS2h 0.547
Legend
Transferase
Epimerase
Other
Regulator
Phenazine core
Transport/Resistance
Terpene/terpenoids/isoprenoid
Oxidases/Reductase
Methytransferase
PKS/NRPS/chain-elongation/AMP/PPE-binding
Dehydrogenase
Unknown
B
Genome name: Rhizobium leguminosarum bv. trifolii CC283b
Scaffold : Rleg11DRAFT_RLJ.1
79,101
104,060
*
Alpha-proteobacteria
Gamma-proteobacteria
Actinobacteria
No hit in IMG database

FIG 7 Diversity of architectures of phenazine biosynthetic gene clusters. (A) Architecturally unique BCs containing all core genes of phenazine biosynthesis were sorted based on similarity to the *Rhizobium leguminosarum* phenazine gene cluster (blue box), which were calculated by using a modification of the average nucleotide identity approach (ANI) (38). Amino acid sequences of proteins encoded in the BC were used to identify bidirectional best hits (BBH) using USEARCH (39). Additionally, amino acid similarity was evaluated instead of identity. For a set of BBH proteins, the average amino acid similarity was calculated in addition to the alignment fraction (AF). The similarity score was calculated as the product of these two values for the *R. leguminosarum* BC (AAS*AF). Phylum classifications are abbreviated as follow: ACT, *Actinobacteria*; AP, *Alphaproteobacteria*; BP, *Betaproteobacteria*; GP, *Gammaproteobacteria*. Phenazine clusters that have been previously studied experimentally are underlined. (B) Evidence for the horizontal transfer of the *Rhizobium leguminosarum* phenazine BC (box). A BLASTp search was performed with each protein in and around the BC as the query against all proteins in the IMG database. Each gene is colored (see legend) based on the phylogenetic class of its top hit (see Table S1 in the supplemental material). Genes marked with an asterisk are transposases.

Most importantly, rhizobia are excellent root colonizers and as such they are great candidates for use in biocontrol applications. To this end, investigators have attempted to engineer a phenazine cluster from *Pseudomonas* into *Rhizobium etli* USDA9032 (31). Although the engineered strain produced the recombinant phenazine, it was unable to produce root nodules. Our discovery of a potential phenazine-producing cluster native in a *Rhizobium* strain isolated from clover root nodules, however, suggests the possibility of combining biocontrol and root-nodulating properties in one bacterial strain. Experiments are under way to investigate the interplay between biocontrol and root nodulation in this *R. leguminosarum* strain, to elucidate the structure of the SM produced by this BC, and to investigate the SM's function, if any, in this host-symbiont relationship.

DISCUSSION

In the last few years, there has been a resurgence of interest in the discovery of natural products, and this resurgence has been fueled by the explosion in the availability of microbial genomic sequences and the expansion of sampling to previously unstudied habitats and environments. However, a very large gap exists between the throughput of sequencing and the rate of discovery of novel pathways involved in secondary metabolism, predominantly because of the absence of tools that facilitate this intellectually and computationally difficult task. Additionally, no public resources exist that allow for the global analysis and comparison of putative biosynthetic gene clusters. These analyses have been accessible only to laboratories staffed with computational biologists and with access to high-performance computing resources.

For the first time, IMG-ABC links information regarding genomic pathways for the biosynthesis of secondary metabolites with chemical structure information on a scale of several thousand data sets. With careful efforts for quality control, it combines the predictive power of state-of-the-art computational tools, such as ClusterFinder and AntiSMASH, with the exhaustive analysis framework offered by the IMG family of systems. This combination delivers a powerful punch, predicting both familiar and novel biosynthetic gene pathways in thousands of cultured isolates, single cells, and metagenomes.

Future work: filling four existing knowledge gaps. (i) Pairwise similarities. By computing pairwise similarities between biosynthetic pathways, we will annotate new BCs demonstrating very high similarity scores (indicating equivalence) with validated biosynthetic pathways. This process will also shed light on previously unknown enzymatic mechanisms.

(ii) Computationally linking SMs with BCs. Traditionally, secondary metabolites were extracted from cultures of isolate organisms and tested for activity, often without any effort to identify the biosynthetic gene cluster responsible for the compound's synthesis. However, in many cases, the genome of the producing organism has since been sequenced and, therefore, it may be feasible to computationally link SMs with BCs. We will explore new approaches to use IMG-ABC's extensive collection of predicted BCs and whole-genome sequences to discover the relevant, but unknown, biosynthetic pathways for organisms known to produce specific compounds.

(iii) A searchable database of predicted SM backbone structures. We will create a searchable database of predicted SM backbone structures for the more than 30,000 BCs that contain either NRPS or type I PKS enzymes (12, 32, 33). This function will be extremely useful for the identification of interesting chemical scaffolds that may be useful starting points for combinatorial chemistry.

(iv) Compatibility and synchronization with the MIBiG centralized database. A major obstacle in collecting data describing experimentally verified BCs and SMs is the absence of a standardized way of reporting these data in public databases. Recently, a consortium of scientists have undertaken the Minimum Information about a Biosynthetic Gene cluster (MIBiG) initiative to provide structured data and high-quality annotations of BCs and the SMs they produce. In the future, we plan to adopt the database structure of MIBiG within IMG-ABC and perform regular synchronizations with the MIBiG centralized database to ensure that the content of experimentally verified data sets in IMG-ABC will continue to grow and reflect the most current and accurate public information.

Conclusion. Computation of biosynthetic gene clusters in newly loaded genomes and metagenomes and their maintenance are now part of the IMG data-loading and annotation pipeline. The growing number of predicted BCs, in conjunction with continuous development of the analysis and search functions available through the system, will ensure that IMG-ABC will always have the latest and most complete publicly available information for the study of secondary metabolism in microbial genomes and metagenomes.

MATERIALS AND METHODS

Collection of gene clusters known to be associated with secondary metabolite biosynthesis. The nucleotide database of NCBI (34) was searched by keywords related to biosynthetic gene clusters. GenBank records with sequences longer than 3,000 bp were retained and manually curated for their encoded SM name, whenever possible. BCs were also retrieved from ClusterMine360 (17) and DoBiscuit (16) irrespective of their length and reconciled with those retrieved from the NCBI database in order to produce a nonredundant list of BCs. The resulting BCs were mapped to isolate genomes and metagenomes in IMG/M based on BLASTN analysis (35) of their nucleotide sequences. Unmapped records were imported into IMG as "genome fragments" (19). Through this process, we acquired 1,332 experimentally verified clusters associated with a GenBank record, most of them imported as "genome fragments." AntiSMASH (version 2.2) was used to assign a type of enzymatic mechanism (BC type) to each gene cluster, when possible (12). Whenever a BC was assigned hybrid or multiple types, the list of types was sorted and concatenated into one text string by using a semicolon separator.

Collection of secondary metabolites and determination of their associations with specific gene clusters. GenBank records of BCs were connected with chemical structures by querying the PubChem Compound database using their associated SM names (26). Exact matches preceded inexact matches. Information retrieved from the PubChem Compound records includes structural and physical descriptors, such the simplified molecular-input line-entry system (SMILES) string (36), the IUPAC international chemical identifier (InChi) (37), molecular weight, etc. Additionally, MeSH Library headings (25) related to classification and pharmaceutical action are also acquired and are represented as "SM Type" and "SM Activity" in our database, respectively. Chemical structures are rendered within IMG by using the NCI/CADD service (http://cactus.nci.nih.gov/).

Computational prediction of putative secondary metabolite biosynthetic gene clusters in genomes and metagenomes. Putative BCs were first identified using ClusterFinder (11) for all isolate genomes and metagenomes in IMG and IMG/M, respectively. ClusterFinder predicts putative BCs based on the Pfam functional annotation of genes available in the integrated context within IMG. BCs predicted in isolate genomes were then classified by the type of biosynthetic enzymes they contain by using AntiSMASH (12). BC predictions were filtered to eliminate BCs that contained less than 6 genes, had a prediction probability of less than 0.3, and were not classified by AntiSMASH to employ a well-defined enzymatic mechanism. Additionally, BC content was analyzed to identify Pfam categories that are not known to be associated with secondary metabolism but are present as positive training features within ClusterFinder. These included Pfam categories such as prophage proteins (PF04883, PF05709, and PF06199), protein secretion systems (PF08817, PF10140, and PF10661), inorganic ion transport proteins (PF02421 and PF11604), and families representing DNA/RNA polymerases, whose presence leads to false-positive BC predictions.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.00932-15/-/DCSupplemental.

Table S1, PDF file, 0.1 MB.

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