Research review paper

Accessing hidden microbial biosynthetic potential from underexplored sources for novel drug discovery

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ABSTRACT

Microbial natural products and their structural analogues have widely used as pharmaceutical agents, especially for infectious diseases and cancer. Despite this success, new structural classes with innovative chemistry and modes of action are urgently needed to be developed to combat the growing antimicrobial resistance and other public health problems. The advances in next-generation sequencing technologies and powerful computational tools open up new opportunities to explore microbial biosynthetic potential from underexplored sources, with millions of secondary metabolites awaiting discovery. The review highlights challenges associated with discovery of new chemical entities, rich reservoirs provided by untapped taxa, ecological niches or host microbiomes, emerging synthetic biotechnologies to unearth the hidden microbial biosynthetic potential for novel drug discovery at scale and speed.

1. History and challenges of microbial natural products for drug development

Microbial natural products (NPs) with intrinsic chemical complexity and intriguing biological profiles, play eminent roles in medicine, agriculture and nutrition (Carroll et al., 2022; Medema et al., 2021; Newman and Cragg, 2020). Historically, microbial NPs have been widely used as antibiotics, anti-tumor compounds and other pharmaceutical agents (Hug et al., 2020; Keller, 2019). In the last five years, a series of NPs or NP-inspired compounds with novel structural scaffolds or modes-of-action (MOA) have been approved for clinical use, crop protection or animal health, such as pleuromutilin-derived lefamulin (antibiotic) and UK-2A-derived fenpicoxamid (fungicide). Undoubtedly, microbial NPs will continue to play important roles in modern medicine, serving as the main source of and inspiration for small molecule drugs to overcome global antimicrobial resistance and other public health problems (Atanasov et al., 2021). Compared with synthetic organic molecules, genetically encoded small molecules offer unique features, including typically higher molecular mass and greater molecular rigidity, and cover a wider area of chemical space for novel drug development (Atanasov et al., 2021). With rapid advances in low-cost and high-throughput DNA sequencing technologies, large-scale microbial (meta) genomic sequencing reveals highly diverse sets of biosynthetic gene

clusters (BGCs) in bacteria or fungi, in which characterized metabolites represent just the tip of the iceberg, with millions of novel NPs awaiting discovery (Hemmerling and Piel, 2022; Kalkreuter et al., 2020; Scherlach and Hertweck, 2021).

On the contrast, we are experiencing a substantially increased antimicrobial resistance (AMR) crisis and other public health problems. NPs with innovative chemical scaffolds and MOA are urgently needed to tackle the AMR or other disease-posed problems (Lewis, 2021; Miethke et al., 2021). However, discovery of new chemical entities is uniquely challenging mainly due to high rediscovery rates. For example, a systematic search of silent BGCs revealed that *Streptomyces*, the main NP sources, have been overmined and have low chance to produce new drug leads against Gram-negative pathogens (Lewis, 2021). Except for *Streptomyces*, a number of bacterial or fungal genera, including *Bacillus*, *Pseudomonas*, *Aspergillus*, *Fusarium* and *Penicillium*, have been extensively studied for novel microbial NP discovery over the last few decades (Gavriilidou et al., 2022; Robey et al., 2021). In the recent study, less popular microbial taxa, including Myxococcaceae, Nostocaceae, Pleurocapsa, Weeksellaceae, Basidiomycota and Mucoromycota, hold promise as underexploited sources of novel NPs with applications in the pharmaceutical and agriscience industries (Gavriilidou et al., 2022; Robey et al., 2021). The insight will facilitate BGC prioritization for the discovery of new chemical entities. In this review, the hidden microbial

biosynthetic potential from underexplored sources is highlighted and the newest developments on emerging biotechnologies to mine and unearth the biosynthetic "dark matter" are discussed.

2. Underexplored sources for the discovery of novel microbial drugs

Underexplored or less-well-studied sources, including untapped bacterial or fungal taxa, microorganisms from global ecological niches as well as complex microbial communities, have been found to encode highly diverse and chemically distinct NPs, which provide rich and unique reservoirs for novel microbial drug discovery (Hemmerling and Piel, 2022; Scherlach and Hertweck, 2021).

2.1. Untapped bacterial or fungal taxa

By accessing ~170,000 bacterial genomes, a compendium of NP biosynthetic diversity (numbers and types of BGCs and GCFs) in genome-sequenced bacteria was systematically evaluated (Fig. 1a) (Gavriilidou et al., 2022). The study clearly indicated that only ~3% NPs have been characterized and several less-well-studied taxa show high biosynthetic diversity, including Myxococcaceae, Nostocaceae, Pleurocapsa and Weeksellaceae (Gavriilidou et al., 2022). These findings will greatly help overcome one of the main bottlenecks of NP discovery: BGC

prioritization for research. Furthermore, genomic and cheminformatics analyses of 1037 fungi revealed that bacterial and fungal sources represent significantly distinct chemical space and biosynthetic logic (Robey et al., 2021). The interpreted atlas of fungal BGCs also revealed that a large variety of chemical entities in a majority of underexplored fungal taxa awaits discovery (Robey et al., 2021).

2.2. Microorganisms from global ecological niches

With advances in sequencing technologies and computational tools, reconstitution of metagenome-assembled genomes (MAGs) has enabled insights into the biosynthetic potential of global ecological niches, including ocean and glacier (Fig. 1a). In a joint study, Nayfach et al., accessed a genomic catalog of Earth's microbiomes, including >10,000 metagenomes from diverse habitats covering all of Earth's continents and oceans (Nayfach et al., 2021). The authors identified 87,187 putatively novel BGCs and found that the phylum Acidobacteria has an underestimated biosynthetic potential. Moreover, Paoli et al., investigated the biosynthetic diversity of microorganisms in the global ocean (>1000 seawaters samples) and unearthed ~40,000 putatively new BGCs encoded by ~10,000 microbial genomes (Paoli et al., 2022). As a representative case, turbinmicin was identified from marine animal microbiomes, which showed potent activity against urgent-threat multidrug-resistant (MDR) fungal pathogens in mice by a fungal-
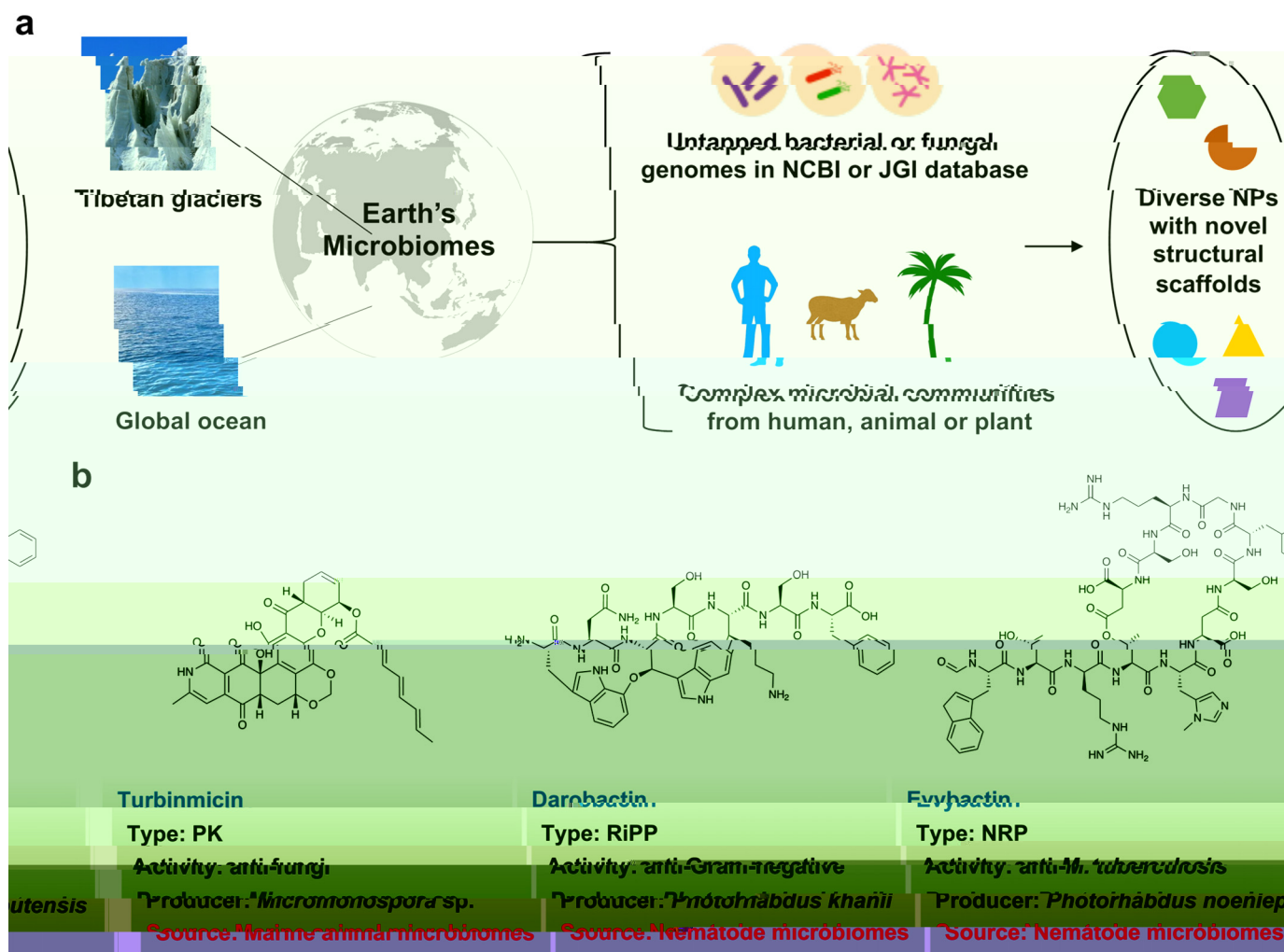

Fig. 1. Underexplored Sources for Microbial Drug Discovery. (a) Unearthing hidden biosynthetic potential from the Earth's microbiomes. Underexplored sources, including untapped microbial taxa, global ecological niches and human, animal or plant microbiomes, open up new opportunities for the discovery of new chemical entities for novel drug discovery. (b) Three structurally diverse NPs with distinct antimicrobial activities from animal microbiomes. Abbreviations: PK, polyketide; RiPP, ribosomally synthesized and post-translationally modified peptide; NRP, non-ribosomal peptide.

specific MOA (Fig. 1b) (Zhang et al., 2022). In another study, Tibetan glaciers, including cryoconite, ice and snow habitats, were found to harbor a diverse range of novel microbial species and provide a unique source to explore BGC novelty and evolution (Liu et al., 2022a, 2022b). Interestingly, although soil ecosystems have been overmined for drug discovery, a number of abundant and phylogenetically bacterial phylums, including Acidobacteria, Gemmatimonadetes, Rokubacteria and Verrucomicobia, has been underestimated, which has a large potential to produce highly diverse chemical scaffolds that warrant further investigation (Crits-Christoph et al., 2018). The studies indicated that metagenomic approaches can overcome the limitation of cultivation techniques and open up opportunities to assess bioactive NPs encoded by environmental DNA.

2.3. Complex microbial communities

Small molecules from soil, ocean or glacier microorganisms can not evolve to function in hosts, such as animals and plants. The symbionts of animal or plant microbiomes have potential to produce new molecules that will function systemically in the inhabited hosts. In fact, complex microbial communities, such as human, animal or plant microbiomes, have been found to encode rich BGCs for new chemistry (Fig. 1a) (Almeida et al., 2021; Bickhart et al., 2022; Scott and Piel, 2019). For example, Bickhart et al., accessed the sleep fecal metagenomes and identified 1400 complete BGCs, most of which are novel (Bickhart et al., 2022). As representative cases, two chemically and mechanistically diverse anti-microbial peptides, including darobactin and evybactin, were isolated from Nematode microbiomes, which exhibited selective activities against Gram-negative bacteria and *Mycobacterium tuberculosis*, respectively (Fig. 1b) (Imai et al., 2019; Imai et al., 2022). As a promising drug lead, darobactin inhibits the outer membrane insertase

BamA by mimicking a -strand (Kaur et al., 2021). The cyclic depsipeptide evybactin targets DNA gyrase when transferred into *M. tuberculosis* by a membrane transporter, BacA, and does not harm human gut microbiomes because gut commensal bacteria lack a BacA-type protein (Imai et al., 2022).

3. Emerging technologies to unearth the hidden microbial biosynthetic potential from underexplored sources

In the last ten years, a wide variety of emerging technologies have been developed for BGC prediction, prioritization and engineering, which significantly facilitate the discovery of bioactive NPs with novel chemical entities. As sequencing data accumulate, BGC prioritization will be critical to discover new class of NPs or diversity among a family of known NPs. The simplest strategy for BGC prioritization is to access NPs′ structures from DNA sequences but only a few classes of NPs, such as non-ribosomal peptides (NRPs) and ribosomally synthesized and post-translationally modified peptides (RiPPs), can be directly predicted. Interested readers are referred to some excellent reviews or articles on BGC prioritization (Bauman et al., 2021; Culp et al., 2022; Panter et al., 2021; Yan et al., 2018). Here, I focus on the discussion of recent technological developments in the last ten years that are enabling discovery of novel microbial NPs from underexplored sources, and highlight selected applications (Tables 1 and 2).

3.1. Computational tools and databases for NP discovery

To facilitate the discovery of microbial NPs, different computational tools and databases have been summaried in recent review papers (Chevrette et al., 2021; Hemmerling and Piel, 2022). Here a number of widely used tools and databases in the field of microbial NPs have been

Table 1
Computational tools and databases for microbial NP discovery.

Function	Name	Link	Description	Reference
BGC prediction	antiSMASH 6.0	https://antismash.secondarymetabolites.org/	A webserver for detecting and characterizing of microbial BGCs	Blin et al., 2021
	PRISM 3	http://magarveylab.ca/prism/	A computational tool for predicting bacterial NPs′ structures	Skinnider et al., 2017
	gutSMASH	https://gutsmash.bioinformatics.nl/	A webserver for identifying primary pathways in gut microbiota	Pascal Andreu et al., 2021
	ARTS 2.0	http://arts.ziemertlab.com/	An antibiotic resistant target seeker for targeted genome mining	Mungan et al., 2020
	CONKAT-Seq	NA	A workflow for co-occurrence network analysis of targeted BGCs	Libis et al., 2019
BGC novelty	BiG-SCAPE/CORASON	https://bigscape-corason.secondarymetabolites.org/	A tool for analyzing sequence similarity of BGCs and their families	Navarro-Muñoz et al., 2020
	BiG-SLiCE	https://github.com/medema-group/bigslice/	A tool for visualizing the diversity of 1.2 million BGCs	Kautsar et al., 2021a, 2021b
BGC database	MIBIG 2.0	https://mibig.secondarymetabolites.org/	A repository for known microbial NPs′ biosynthetic pathways	Kautsar et al., 2020
	antiSMASH Database v3	https://antismash-db.secondarymetabolites.org/	A database for predicted BGCs from high-quality genomes	Blin et al., 2021
	IMG-ABC v.5.0	https://img.jgi.doe.gov/abc-public/	An integrated microbial genomes atlas of predicted BGCs	Palaniappan et al., 2020
	Big-FAM	https://bigfam.bioinformatics.nl/	A database for predicted microbial BGC families	Kautsar et al., 2021a, 2021b
	ARTS-DB	https://arts-db.ziemertlab.com/	A database for pre-computed antibiotic resistant targets	Mungan et al., 2022
	ClusterMine360	http://www.clustermine360.ca/	A database of microbial PKS/NRPS biosynthesis	Conway and Boddy, 2013
	MycoCosm	http://jgi.doe.gov/fungi	An interactive web-based portal for fungal genomics resources	Grigoriev et al., 2014
NP database	Natural Products Atlas 2.0	https://www.npatlas.org/	A database for microbially-derived known natural products	van Santen et al., 2022
	SuperNatural 3.0	http://bioinf-applied.charite.de/supernatural_3	A database of natural compounds and their derivatives	Gallo et al., 2023
	NPASS	http://bidd2.nus.edu.sg/NPASS/	A database of natural product activity and species source	Zeng et al., 2018
	StreptomeDB	http://132.230.56.4/streptomedb2/	A resource of natural products produced by streptomycetes	Klementz et al., 2016
	GNPS	http://gnps.ucsd.edu/	A resourece of tandem mass (MS/MS) spectrometry data	Wang et al., 2016

discussed about their advantages and limitations of usage. A variety of powerful computational tools, such as antiSMASH 7.0, gutSMASH and ARTS 2.0, have been developed for prediction of BGCs from gut microbiota or other microbial sources (Blin et al., 2023; Mungan et al., 2020; Pascal Andreu et al., 2021). Particularly, antiSMASH 7.0 is currently the most widely used computational platform to detect and characterize up to 81 BGC types in microbes. Meanwhile, the accuracy of chemical structure prediction in antiSMASH 7.0 has further been improved, especially for non-ribosomal peptides. However, there is still a big obstacle to optimize the structural prediction accuracy of other NP types, including polyketides (PKs) and terpenes. Meanwhile, a panel of unsupervised algorithms such as BiG-SCAPE/CORASON and more recently BiG-SLiCE, have been developed to analyze sequence similarity of BGCs and gene cluster families (Kautsar et al., 2021a, 2021b; Navarro-Muñoz et al., 2020). Nowadays, both algorithms have been widely used to identify novel features within and between taxonomic lineages by clustering BGCs into gene cluster families (GCFs). Noteworthy, high-quality genomic and chemical databases are still required for these unsupervised platforms. Furthermore, a bioinformatic workflow termed CONKAT-seq was developed for co-occurrence network analysis of targeted DNA sequences, which allows scalable discovery of novel natural compounds from soil microbiomes (Libis et al., 2019). The CONKAT-seq approach can be used to rapidly detect and characterize novel NRP or PK gene clusters, but requires to be further optimized for other BGC types in the future.

Besides these computational toos discussed above, repositories for BGCs encoded by known NPs (e.g., MIBiG 2.0) or unknown chemical entities (e.g., antiSMASH database v3, IMG-ABC v.5.0, BiG-FAM and ARTS-DB), have been   in   of BGCs (Blin et al., ; 21 2 Kautsar et al., ; 20 2 Kautsar et al., ; 21a, ; 21b 2 Mungan et al.,   2 Palaniappan et al., ; 20 ;). For example, the BiG-FAM database groups homologous BGCs int   Cluster Families (GCFs) and includes 29,955 GCFs covering the global diversity of 1,225,071 BGCs, which provides new insights int   accessing the novelty of BGCs by mapping   and taxonomic diversity (Kautsar et al., 2021a, 2021b). ARTS-DB,   for antibiotic resistance targets from >70,000 genomes and metagenomes, opens up new possie   for rapid   mining, including BGC proximity and   of housekeeping genes (Mungan et al., 2022). In addition, the Natural Products Atlas 2.0,   database

of known microbial NP structures, has been released, which contains the structural and content changes of 32,552 compounds as well as taxonomic   for all bacterial or fungal   van Santen et al., 2022).   a larger database of NPs and derivatives, SuperNatural 3.0, was released, which contains 449,058 compounds with their physicochemical and structural information (al., 2023). These powerful computational tools and databases, which have been listed in Table 1,   up new opportunities   explore hidden microbial biosynthetic potential from underexplored sources.

3.2. Activating silent BGCs from undomesticated microorganisms

Generally, underexplored natural compound producers, such as most of Gram-negative proteobacteria, pnaerobic bacteria pnd filamentous fungi, are genetically   A large variety of novel enabling technologies is required to be developed to access their biosynthetic potential, including species-specific genome editing tools for in situ BGC activation and high-efficient BGC engineering approaches for heterologous expression.

3.2.1.   in native producers
In situ BGC activation mainly relies on high-efficient genome editing for promoter engineering. 2

2022). Considering that it is a direct route for NP discovery by in situ genetic engineering but >90% BGCs are silent or cryptic under standard laboratory conditions (Rutledge and Challis, 2015; Walsh and Fischbach, 2010), more general synthetic biology approaches are still needed, especially for genetically intractable strains.

3.2.2. Heterologous expression of native or refactored BGCs

In the last five years, a variety of versatile surrogate heterologous hosts has been developed for novel drug lead discovery from distinct bacterial or fungal taxa (Liu et al., 2022a, 2022b; Meng et al., 2022; Roux and Chooi, 2022). For example, genome-reduced *Schlegelella brevitalea* classis exhibited alleviated cell autolysis and was better than two commonly used Gram-negative chassis *Escherichia coli* and *Pseudomonas putida* for production of cryptic metabolites from proteobacteria (Liu et al., 2021). Meanwhile, a variety of Cas12a-assisted approaches has been developed for targeted and precise BGC cloning, including CAT-FISHING and CAPTURE (Enghiad et al., 2021; Liang et al., 2022). For example, Enghiad et al., used CAPTURE to clone 47

and synthesize 20 candidate AMPs within 48 days (Das et al., 2021). Notably, Torres et al., used the similar algorithm to identify 2603 encrypted peptide antibiotics from the human proteome (Torres et al., 2022). These studies open up new routes for the discovery of candidate antibiotics from host or environmental microbiomes (Fig. 3).

In 2022, based on natural language processing and deep learning, Ma et al., developed a clever AI approach to access complex human metagenomic information (Fig. 3) (Ma et al., 2022). They first combined different deep-learning-based natural language processing models to construct an optimized pipeline for AMP discovery. Then, 4409 qualified bacterial genomes from human microbiomes were screened using the advanced AI pipeline and >20 million predicted AMPs were found from non-redundant sORF sequences. By cross-checking with the available metaproteomic data, 2349 candidate AMPs were eventually identified. Combined with relative abundance, gene expression and association with selected bacterial taxa, 241 peptides were finally selected for chemical synthesis. A total 216 peptides were successfully synthesized and 181 of these showed antimicrobial activity with a high hit rate. More importantly, they found that most of these synthesized AMPs show low identities of <40% to previously conventional peptides, which provide novel chemical entities for drug discovery. Among all 181 AMPs, 11 were the most potent effective against drug-resistant pathogens and three showed high anti-infective efficacy and low toxicity in a mouse lung infection model (Fig. 3) (Ma et al., 2022).

Similarly, Linda et al., used the classifier model design to discover two AMPs from a rumen microbiome metagenomic dataset, which were also active against methicillin-resistant *Staphylococcus aureus* (MRSA) in a *Galleria mellonella* infection model (Oyama et al., 2022). Collectively, the AI driven chemical synthesis of linear peptides or small proteins presents a powerful methodology for rapidly accessing novel antibiotics from complex host or environmental microbiomes and has a promising potential to replenish our depleted arsenal. One possible limitation of AI-driven AMP discovery is their propensity toward biases. More robust training sets and experimentally validated models will further enhance success rate and decrease sequence biases. Additionally, AI-derived discovery pipeline requires to be further optimized and extended to access other structural classes of microbial NPs.

3.4. De novo and targeted syn-BNP for antibiotic discovery

Nonribosomal peptides (NRPs) show high chemical and mechanistic diversity, which have been widely used as lead compounds for drug development, such as daptomycin and colistin (Liu et al., 2019). Thanks to the deep understanding of biosynthesis for known NRPs, chemical structures of a large variety of NRPs can be predicted with high confidence (Süssmuth and Mainz, 2017). This provides an alternative route to access BGC-encoded products via bioinformatics prediction followed by chemical synthesis, that is a synthetic-Bioinformatics Natural Product (syn-BNP) approach. Here, I discuss some representative examples that demonstrate the utility of this new methodology with an emphasis on the targeted syn-BNP approach to unlock biosynthetic diversity of a panel of known antibiotics or new antibiotic family.

3.4.1. De novo syn-BNP for random discovery of antibiotics

In 2016, the Brady group first accessed NRP BGCs from human microbiomes using the syn-BNP approach and humimycins from 25 synthesized linear syn-BNPs were found to be active against MRSA by inhibiting lipid II flippase (Chu et al., 2016). Then, this approach was extended to explore cyclic peptides for novel antibiotic discovery (Chu et al., 2020). 157 designed peptides with different cyclization modes were successfully synthesized and nine inhibited bacterial growth by diverse modes of action (Chu et al., 2020). In 2021, using penicillin binding protein-like cyclase as target, Hostetler et al., also developed a library of predicted head-to-tail cyclic peptides encoded by >500 NRP BGCs from underexplored actinobacteria and identified 14 antibacterial compounds (Hostetler et al., 2021). The traditional NP discovery paradigm that involves in fermentation of producers is laborious and time-consuming to identify and isolate metabolites, and even the best activation strategies can only unlock a small fraction of silent BGCs. On the contrast, the *syn*-BNP approach completely skips the paradigm and opens up new opportunities to unlock >90% silent BGCs under standard laboratory conditions.

3.4.2. Targeted syn-BNP for the discovery of menaquinone-binding antibiotics

Although the de novo syn-BNP approach can access novel peptides, some obvious obstacles, including the low success rate and moderate activity of syn-BNPs, are required to be overcome. Recently, a targeted syn-BNP approach was developed in the Brady group, which can be used to discover more potent compounds, including congeners of known AMPs and new antibiotic families (Li et al., 2022; Wang et al., 2021; Wang et al., 2022a, 2022b, 2022c). They developed a predicted NRP database that contains >36,000 linear peptides from >10,000 bacterial genomes (Li et al., 2022). Using the predicted database combined with sequence tag-based metagenomic mining, Li et al., developed a novel NP discovery approach, that is motif search of predicted structures, and identified six novel menaquinone-binding antibiotics (MBAs) from untapped Gram-negative bacteria or soil metagenomes (Fig. 4a). Furthermore, MBAs were firstly found to show potent activity against *Mycobacterium tuberculosis* by targeting menaquinone in bacterial membrane (Li et al., 2022). Identifying NPs with a desired MOA by searching for a substructure among a database of predicted structures
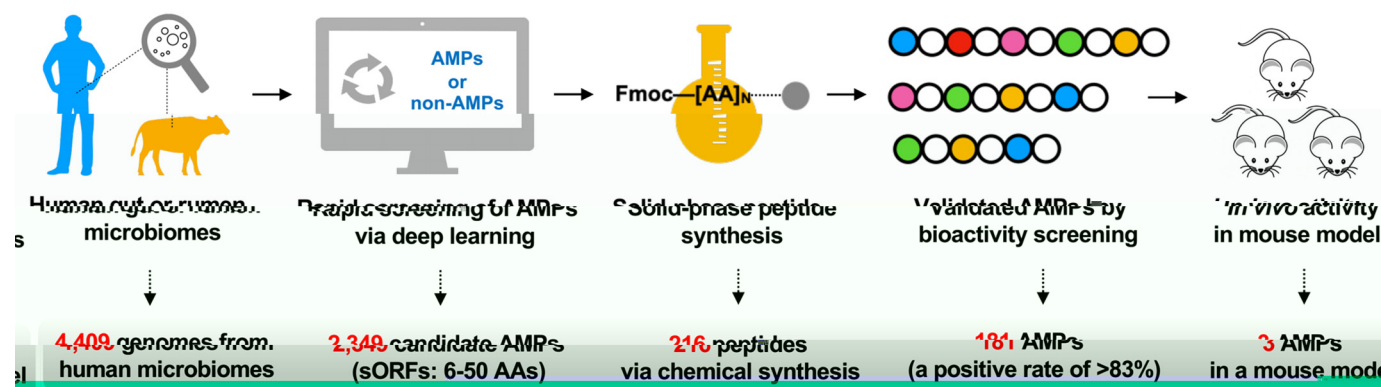

Fig. 3. AI-derived AMP Discovery. To develop a powerful AMP prediction pipeline, a panel of neural network models are required to be builded and optimized by collecting sequences of known AMPs to build training and test sets. Then, promising candidates are synthesized and tested against MDR bacteria in an animal model. A representative work from Ma et al. was shown for searching for novel AMPs from human gut microbiomes using deep learning and natural language processing. Abbreviations: AMP, anti-microbial peptides; sORFs, small open reading frames.
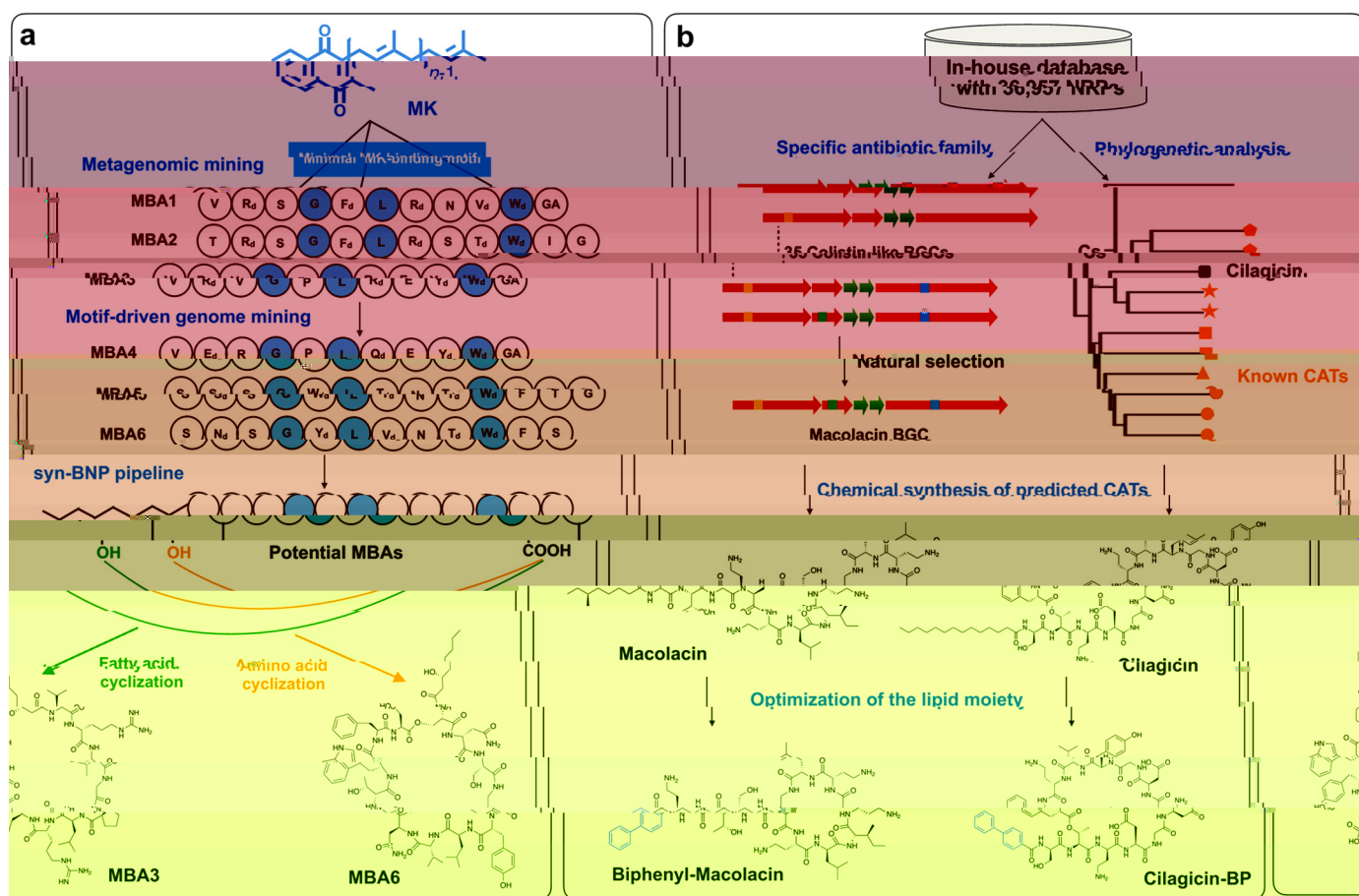
Fig. 4. Targeted *syn*-BNP for Antibiotic Discovery. (a) (Meta)genomic mining of menaquinone-binding antibiotics (MBAs). Two orthogonal pipelines, including sequence tag-based metagenomic mining and motif search-based genome mining, were used for MBA discovery. Linear MBAs were cyclized through the -hydroxyl of the fatty acid or through a nucleophilic amino acid side chain. (b) Targeted genome e   cationic lipopeptides (CATs). Based on the concept of natural selection, e   colacin was identified by searching for colistin congeners to overcome its resistance. Phylogenetic analysis of Cs domain sequences of known or potential was used to guide the discovery of cilagicin. To further   in   efficacy, biphenyl lipid moiety was introduced into e   colacin or cilagicin. Abbreviations: syn-BNP, synthetic-Bioinformatics Natural Product; NRPs: non-ribosomal peptides; Cs: condensation start domain.

provides a generalizable and high-throughput approach for discovery of novel bioactive NPs.

3.4.3. Targeted syn-BNP for the discovery of cationic lipopeptide antibiotics

Cationic lipopeptides (CATs) are generally capable of penetrating the outer membranes of Gram-negative bacteria and interacting with different anionic intracellular targets, which show potent activity against a large variety of MDR Gram-negative pathogens (Liu et al., 2019). Intriguingly, a few CATs (e.g., Lysocin E and WAP-8492A2) have been proven to possess narrow-spectrum activity against Gram-positive pathogens (Yue et al., 2022). The diverse antimicrobial activities make CATs an appealing class of antibacterial agents for in-depth (meta) genomic mining. By systematically analyzing 5585 complete bacterial genomes, Li et al., firstly identified two Gram-negative-active CATs, brevicidine and laterocidine (Li et al., 2018). Instead of the time-consuming fermentation, the Brady group recently used the targeted *syn*-BNP approach to rapidly discover two novel CATs with unique features (Fig. 4b) (Wang et al., 2022a, 2022b). In the first study, to overcome colistin resistance, they set out to identify BGCs that encode the colistin congeners from the predicted NRP database (Wang et al., 2022a). Totally, 35 colistin-like BGCs were identified and the predicted product encoded by one of these BGCs, macolacin, was synthesized and found to be active against a variety of colistin-resistant clinical isolates. (Fig. 4b) (Wang et al., 2022a). In the second study, via phylogenetic analysis of condensation start domain, the same group identified a

distinct CAT BGC that encodes cilagicin. Cilagicin caused cell death by directly binding to both undecaprenyl phosphate (C55−P) and unde-caprenyl pyrophosphate (C55-PP). Notably, they did not detect resistance to cilagicin due to its dual MOA (Fig. 4b) (Wang et al., 2022b). The targeted syn-BNP approach provides an inspirational interdisciplinary and high-throughput pipeline for future antibiotic discovery to overcome antimicrobial resistance as well as high-throughput discovery of other drugs.

4. Concluding remarks

The study of microbial NPs can not only help to better understand their functions in native environment, but also discover more drug leads with novel chemical entities or distinct modes of action. With biological and technological advances in the last two decades, the cost of DNA sequencing has been dramatically decreased and the capacity of sequencing complex samples has been significantly improved. Thanks to this unprecedented access to (meta)genomes of unconventional microbial taxa and global microbiomes, we are witnessing a renaissance in natural product discovery for novel drug development. Major bottlenecks in accessing the hidden biosynthetic potential are being overcome by the enabling technologies discussed in this review (Table 2) and future increasing developments.

Several important questions remain and require to be addressed in the near future. For example, how to develop more effective strategies of

BGC prioritization for the discovery of new chemical entities? Big data-driven computational tools (e.g., BiG-SCAPE/CORASON) to analyze BGC novelty at scale and targeted (meta)genomic mining of a type of NPs (e.g., motif search of predicted NP database) could be efficient solutions for BGC prioritization. Will the cost of gene synthesis be decreased significantly in the near future? If so, it will be available to refactor large-size BGCs (>100 kb), such as non-ribosomal peptide and polyketide biosynthetic pathways. Additionally, how to improve prediction accuracy and decrease sequence biases of artificial intelligence (AI) driven discovery of antimicrobial peptides? Specially, is it possible to extend the AI technique or the syn-BNP approach to access other classes of NPs besides bioactive peptides from untapped microbial sources? Except for peptides, building blocks recognized by acyl-transferase (AT) domains in type I polyketides (PKs) and hybrid polyketide-non-ribosomal peptide (PK-NRP) compounds could be predicted based on ATSignature or Minowa software with low confidence. However, it is expected that structures of PKs or hybrid PK-NPR molecules could be accurately predicted along with rapid development of AI and computational tools. Nevertheless, considering the efficiency and breadth of new (meta)genomic mining approaches and given the increased extent of underexplored sources, I believe that NP-based drug discovery and development will continue to make major contributions to human health.

Declaration of Competing Interest

The author declares no competing financial interest.

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