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Natural products discovery and potential for new antibiotics

Olga Genilloud

Microbial natural products have been one of the most important sources for the discovery of potential new antibiotics. However, the decline in the number of new chemical scaffolds discovered and the rediscovery problem of old known molecules has become a limitation for discovery programs developed by an industry confronted by a lack of incentives and a broken economic model. In contrast, the emergence of multidrug resistance in key pathogens has continued to progress and this issue is compounded by a lack of new antibiotics in development to address most of the difficult to treat infections.

Advances in genome mining have confirmed the richness of biosynthetic gene clusters (BGCs) in the majority of microbial sources, and this suggests that an untapped chemical diversity is waiting to be discovered. The development of new genome engineering and synthetic biology tools, and the implementation of comparative omic approaches is fostering the development of new integrated culture-based strategies and genomic-driven approaches aimed at delivering new chemical classes of antibiotics.

Address
Fundación MEDINA, Avda Conocimiento 34, 18016 Granada, Spain

Corresponding author:
Genilloud, Olga (olga.genilloud@medinaandalucia.es)

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Introduction

The current spread of antimicrobial resistance (AMR) represents one of the most serious threats to human health worldwide. Serious infections caused by antibiotic-resistant bacteria are no longer responding to available treatments and can rapidly develop resistance to the antibiotics last resort. Multidrug resistant pathogens are a global priority for the World Health Organization and a priority list of antibiotic-resistant bacteria has been published, recommending research and development of effective drugs for the treatment of these infections [1]. A recent study monitoring twenty critical species with different patterns of acquired resistance has defined a high priority tier of pathogens that should be urgently addressed given the lack of novel compounds to combat AMR: carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*; carbapenem-resistant and third-generation cephalosporin-resistant Enterobacteriaceae; vancomycin-resistant *Enterococcus faecium* and methicillin resistant *Staphylococcus aureus*; as well as pathogens from community acquired infections including clarithromycin-resistant *Helicobacter pylori*, and fluoroquinolone-resistant *Campylobacter* spp., *Neisseria gonorrhoeae*, and *Salmonella typhi* [2•]. Despite this growing clinical need, the withdrawal of large pharmaceutical companies from antibiotic research due to a broken economic model has resulted in the absence of new classes of antibiotics reaching the market, leaving antibiotic discovery and development to academic and small biotech companies [3•]. The absence of pull incentives and limited funding instruments de-risking antimicrobial development for small biotech companies is creating the perfect storm just when a coordinated effort is urgently needed to guarantee an early pipeline of new antibiotics [4•].

Current antibiotic discovery strategies

Microbial natural products provide the origin of most classes of antibiotics currently in clinical use and they continue to represent privileged structures resulting from natural evolution. They represent one of the most important sources of chemical diversity for the discovery of new molecules compared to the lack of success of synthetic molecules selected from target based *in vitro* screens, or those arising from rational drug design based on ligand binding which often lack the physiochemical properties to penetrate bacterial membranes [5–7]. Traditionally, phenotypic screening approaches have been the most effective for identifying compounds with the empiric ability to prevent cell growth *in vitro*, but the rediscovery problem of known compounds has limited the continued investment in natural products discovery programs. Whole cell, target-based assays represent a recent paradigm shift in antibiotic discovery. For example, approaches such as the *S. aureus* fitness test is based on silencing essential gene expression and selectively decreasing the levels of targeted gene products, sensitizing the strains to an inhibitor that targets the depleted gene product. These approaches enabled the discovery of new classes of antibiotics such as the FabF inhibitor platensimycin, as well as kibdelomycin, a new structural class of bacterial DNA gyrase inhibitors [8,9]. Recent

phenotypic screens developed for readouts beyond growth inhibition have permitted the phenotypic fingerprinting of sublethal doses of compounds based on the quantification of changes in cell features with specific fluorescent stains for membranes, DNA and membrane permeability [10]. The integration of this strategy in a multiparametric, high-content screening approach based on monitoring the lowest effective dose determining a phenotypic change has permitted the investigation of low potency hits from industrial collections and the identification of novel antibacterial compounds with differential modes of action at concentrations below the MIC [11••].

Compounds disrupting critical protein–protein interactions in bacteria may represent another option for the discovery of new antibiotics [12•]. Protein–protein interactions in bacteria coordinate many bacterial physiological processes involved in the regulation of gene expression, DNA replication, signal transduction, and virulence. These protein–protein interaction networks, which constitute the bacterial interactome, have been studied for some bacteria and will be critical to identify potential points of intervention in clinically relevant pathogens and drug targets for antibacterial discovery [13,14]. Natural products have excellent structural characteristics to modulate these interactions, due to their molecular size and three-dimensional complexity. Griselimycin, an inhibitor of M. tuberculosis in the low micromolar range, has been shown to bind to the bacterial DNA polymerase sliding clamp DnaN, the ring-shape protein securing DNA polymerases to the DNA template. This direct molecule interaction interferes with essential DNA polymerase and DNA repair activities and has validated the sliding clamp DnaN as a druggable antibacterial target [15].

New efforts are also being directed at rescuing old antibiotics, or testing new drug combinations, an approach supported by the previous success of compounds engaging multiple targets and leading to synergistic bactericidal effects and decreased resistance rates [16]. Adjuvant molecules have been targeted at enzymes mediating drug resistance or antibiotic efflux systems involving, for instance, the cytoplasmic membrane major facilitator superfamily spanning membrane systems. This strategy is currently being revisited as an alternative approach to improve efficacy, expand the antimicrobial spectrum and overcome the emergence of resistance, but their preclinical development is also challenged by the complex pharmacology required of a successful combination of antibiotic actions [17•]. The generation of hybrid antibiotics combining active domains in one single molecule have been explored as alternative solution, and Cefilavancin, the combination of a glycopeptide with a cephalosporin, provides an example currently in clinical development [3•]. Targeting non-essential processes, and interactions between microbes and the host, are also at the center of alternative strategies to develop new compounds to overcome resistance and rescue the activity of last resort antibiotics. Anti-virulence agents lacking bactericidal activity but affecting bacterial quorum sensing have been proposed as a viable strategy to address antibiotic resistance with low selective pressure [18].

Opportunity for microbial natural products

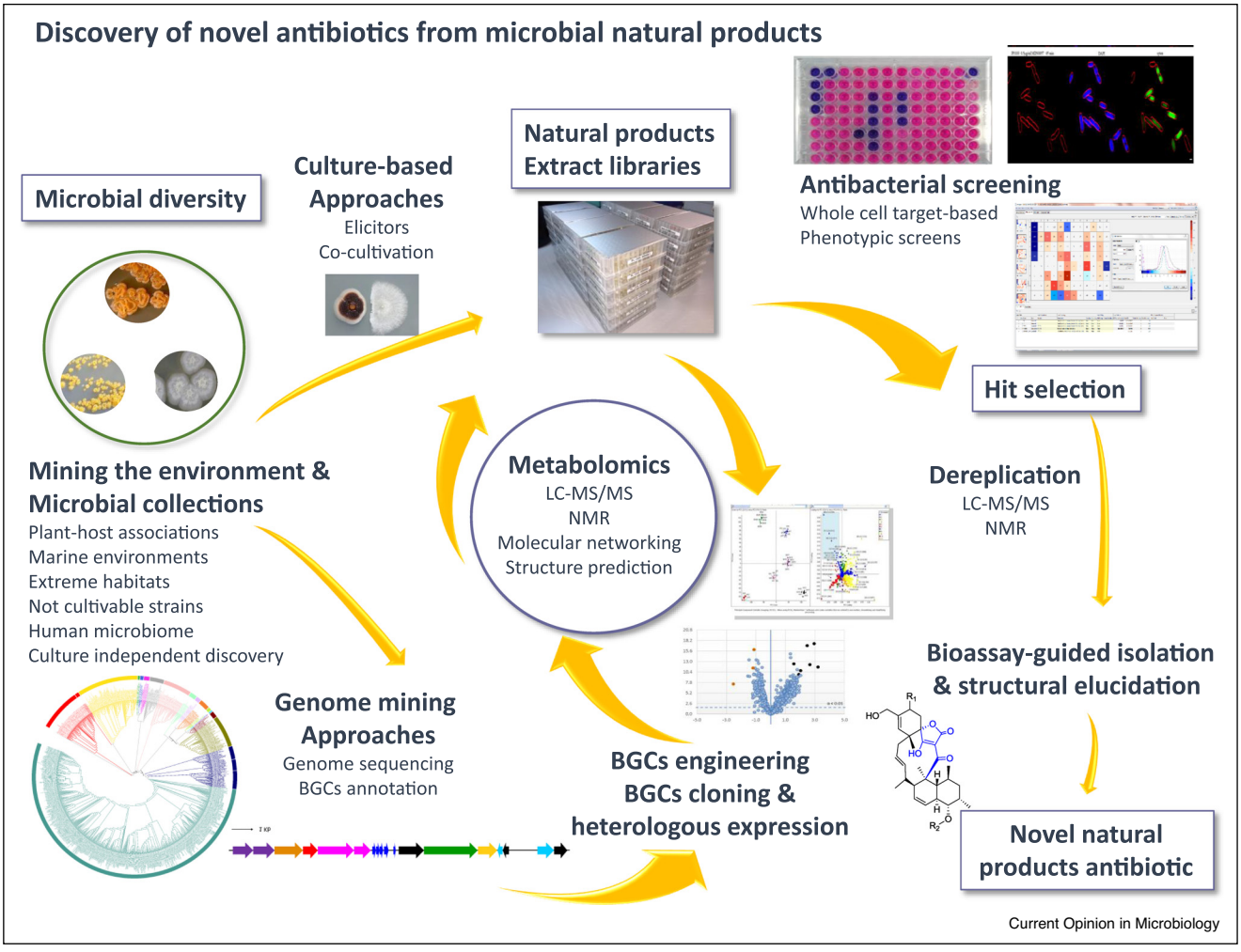
Current antimicrobial research with natural products is confronted with the challenge of identifying new structural scaffolds, that act on new targets, and can evade cross-resistance mechanisms while overcoming the problems of membrane permeability and efflux pumps. A retrospective analysis of the chemical space of all published microbial and marine-derived natural products from the period 1941–2015, encompassing a broad array of biosynthetic origins, has shown that known natural product scaffolds occupy a relatively narrow part of the total available natural product-like chemical space indicating a significant opportunity for novel compound discovery [19••,20]. However, at the same time there are serious concerns regarding the limitations of traditional natural products screening approaches to deliver novel compounds, while advances in next generation DNA sequencing have shown us that most of the biosynthetic potential encoded in microbial genomes is not expressed under laboratory conditions and waits to be discovered.

Thus, there is a need to address natural product research from a totally new perspective by combining, in a multidisciplinary strategy, all of the new diversity mining and culture-based technologies with all of the omic-based tools available (Figure 1). Research in natural products biosynthesis has rapidly evolved during the last decade and integrate advances in genome sequencing and genome mining tools as well as in strain engineering, bioinformatics and analytical chemistry tools [21].

Biodiversity of sources and improved cultivation

Exploiting the metabolic diversity of cultured microbial strains from poorly explored habitats remains a very active field with the description of novel clades, previously uncultivated strains, and the production of novel compounds. Recent successes include bioactive molecules obtained from microorganisms isolated from extreme arid areas, pristine caves, plant endophytes and epiphytes, insect microbiomes, a broad diversity of marine ecosystems, and the human microbiome [22–30]. Recent genome-based analysis of anaerobes has identified an unexpected natural products biosynthetic potential [31]. Furthermore, metagenomic assessment is revealing the dynamics of microbial populations in these environments and identifies a potential role for antimicrobial

Figure 1



Current multidisciplinary approach used in the discovery of microbial natural products antibiotics: this integrates new genome diversity mining and culture-based technologies with all the analytical omic-based tools, traditional bioactivity screening and the bioengineering of biosynthetic gene clusters.

natural products within these microbial communities associated with virulence, motility, stress response and defence. Despite extensive isolation efforts, most members of microbial communities cannot be cultivated under laboratory conditions and identifying and isolating new strains producing novel antibiotic compounds remains a challenge. Different methods were developed to grow previously uncultured microorganisms by exposing cells to growth factors and environment signalling molecules. *In situ* cultivation methods using diffusion chambers or iChip technologies have been developed for enriching in previously uncultivated bacteria and improving growth recovery. These efforts contributed to the isolation of several new isolates such as *Elephteria terrae*, *Nocardia* sp. and *Lentzea kentuckyensis* and the discovery of the new antibiotics such as teixobactin, neocitreamicins, and lassomycin respectively [32–34].

Microbial interactions: elicitors and co-cultivation

Culture-based approaches have been one of the most commonly used methods for the identification of new compounds and several strategies have been developed to activate silent or poorly expressed BGCs. These methods are also required for strains recalcitrant to genetic manipulation meaning the biosynthetic pathways cannot be engineered, or where access to genome sequence data is limited. Co-cultivation takes advantage of natural microbial interactions occurring in the environment to trigger the production of antibiotics. Small concentrations of diffusible signalling molecules or secondary metabolites produced by one of the strains can modulate gene expression, or act as elicitors, to activate silent pathways, whereas in other cases physical cell-to-cell interaction is required for this induction. A good number of interesting novel secondary

metabolites have been identified from co-cultivated microorganisms. This is the case of alchivemycin A and B, produced after co-culturing a *Rhodoccocus* strains with another strain of *Streptomyces*. Similarly, arcyriaflavin E or ciromicins were obtained after co-culturing different Rhodoccoci with species of *Tsukamurella* and *Nocardiopsis*, and Keyicin resulted from the cocultivation of a *Micromonospora* strain with *Rhodococcus* strain [35–37]. A broad range of small molecule elicitors, including subinhibitory concentrations of antibiotics, have been used successfully to perturb biological systems and signalling pathways leading to activation of silent or poorly expressed [38,39]. The development of the HiTES strategy (high throughput elicitor screening) for eliciting and detecting the cryptic metabolome in bacteria in conjunction with imaging mass has enabled the analysis of the induced metabolome in hundreds of different conditions [40–42,43•]. The Bioactivity-HiTES approach combines HiTES with bioactivity screening to directly identify bioactive cryptic metabolites from the different induced metabolome conditions. The description of the Gram-negative active antibiotics taylorflavins A and B and the new lanthipeptide cebulantin are recent examples that validate this approach [44,45].

Genome targeted antibiotic discovery and the multi-omics approach

The explosion of microbial genome sequences has illuminated the breadth of untapped biosynthetic diversity. New and improved tools for BGC identification and annotation such as AntiSMASH, RODEO or eSNAPD are enabling the search for defined scaffolds and the prediction of compound structures from biosynthetic pathways when signatures are available to be detected by rule-based bioinformatic tools [46,47,48•,49•,50]. The MIBiG and IMG-ABC databases are standardizing experimental annotation data for BGCs to enable comparative and functional analyses [51,52•]. Nevertheless, linking genome annotation and metabolite identification remains a challenge, and tools such as MAGI have been developed to help link metabolomics and protein gene sequence data [53]. Additionally, genome handling tools are enabling whole genomic bacterial artificial chromosome libraries to be built from large fragments of genomic DNA, that can be used in high throughput functional screening approaches to identify previously unidentified BGCs encoding for new antibiotics [54]. The development of new optimized approaches for cloning and heterologous gene expression, as well as the targeted engineering of these pathways, are bringing new synthetic biology tools to the field of natural products discovery. New genetic engineering tools enable us to modify metabolic pathways, inactivate transcriptional repressors, over express pathway-specific activator genes, and alter metabolic fluxes [55,56•,57,58]. The 'cracking the code' approach involves identifying the right combinations of regulatory elements and transcription factors that regulate a BGC in order to identify elicitor signals that can activate silent pathways [59]. The development of optimal ribosomal binding sequences and strong terminators is also offering new possibilities for modulating gene expression and metabolic engineering [60•].

The integration of omics and genome engineering approaches are therefore supporting the analysis of the biosynthesis of microbial secondary metabolites and consolidating an integrated strategy for the discovery of new antibiotics from a broad range of different perspectives [61]. To overcome the major challenge of the identification of novel bioactive molecules within complex metabolomic profiles, new dereplication and identification approaches have been being developed based on MS/MS patterns and molecular networking, and NMR based metabolomics. The new NMR techniques are leveraging chemical shift patterns to predict stereochemical relationships and rapid determination of connectivity [62,63]. Proteome-mining is linking metabolites to biosynthetic enzymes and enables the correlation of expression profiles for biosynthetic enzymes to the metabolome of the producing strains and is based on statistical analysis of strains cultured under different conditions [64,65]. The combination of metabolomics with genome analysis or 'metabologenomics' has enabled the discovery of new natural products [66,67]. New genome scale metabolic models are opening new avenues to study the primary and secondary metabolism interactions and for the first-time proposing gene manipulations for overproduction of natural products [68].

Conclusions

Advances in the field of natural products clearly show that multi-disciplinarity and the integration of multiple new technologies are required to develop and coordinate natural products research, and to fully exploit the still untapped chemical diversity of microbial communities for the discovery of new antibiotics. Although the potential of natural products as privileged molecules for antimicrobial development is well recognized, the challenge of discovering new chemical scaffolds is huge: and they must have the appropriate physiochemical characteristics to overcome the many barriers to entering a cell, and then reach therapeutically relevant targets, in the most critical multidrug resistant pathogens. The efficient integration of culture-based approaches and genome driven synthetic biology platforms will be needed in order to deliver the new preclinical candidate molecules that the antimicrobial research field urgently requires to address the innovation gap and fill the antibiotic pipeline.

Conflict of interest statement

Nothing declared.

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