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Current developments in antibiotic discovery

Global microbial diversity as a source for evolutionary optimized anti-bacterials

Julian D Hegemann[1,2], Joy Birkelbach[1,2], Sebastian Walesch[1,2] & Rolf Müller[1,2,3,*]

See also: **S Walesch *et al*** (January 2023)

Antibiotics have profoundly revolutionized medicine and the health care sector since Alexander Fleming first identified the bacteria-killing properties of penicillin. The following golden age of antibiotic discovery from the 1940s to the 1960s yielded a variety of microbial natural products that rendered previously life-threatening bacterial infections efficiently curable. Owing to their comparably low manufacturing costs and broad accessibility, antibiotics quickly and considerably helped to increase average life expectancy and well-being of many humans. Antibiotics have not been limited to treating human diseases but have also been used widely in animal husbandry to facilitate close-quarter factory farming and to promote animal growth. Apart from the ethical implications of industrialized lifestock farming, it has greatly contributed to the rise and spread of antibiotic resistance in microbial communities.

Given these enormous benefits, it is, in retrospective, not surprising that reckless administration and overuse became rampant (Antimicrobial Resistance Collaborators, 2022). As a result, many pathogens have now become resistant against antibiotics. This unsettling development unfortunately coincides with many big pharmaceutical companies cutting funding for or even completely withdrawing from research and development of novel antibiotics. There are numerous reasons for this trend including the high costs of clinical trials combined with a lack of financial incentives for releasing new antibiotics onto the market. Moreover, the business model has changed since any new antibiotics will be restricted to emergency use in the clinic, which drastically reduces potential profits.

> *"This alarming combination of spreading antibiotic resistance and decreasing innovation is casting a looming antibiotic-resistance crisis."*

This alarming combination of spreading antibiotic resistance and decreasing innovation is casting a looming antibiotic-resistance crisis. It could lead to a worst-case scenario, in which antibiotic resistance is so common that most bacterial infections will again become untreatable by drugs and humanity would be thrown back into the pre-antibiotic era. An estimated 0.9–1.7 million deaths per year are already attributable to antimicrobial resistance and, at the current rate of spreading resistance, this number could increase to as many as 10 million by 2050 (Antimicrobial Resistance Collaborators, 2022). However, blaming a lack of investment would be an oversimplification—although developing new antibiotics is a difficult problem, solutions do exist. Even if reduced investments means reduced research and development, we also have to realize that innovations in antibiotic research are now much harder to accomplish and require more funding than during the golden decade of discovery.

One main reason is the diminishing effectiveness of the traditional approaches for discovering antibiotics. Most antibiotics used in medicine are microbial natural products—or derived from such microbial compounds—and were discovered by isolating and cultivating a few types of soil microorganisms and screening culture extracts for antibiotic activities (Newman & Cragg, 2020). This strategy was initially efficient and productive, but progress steadily slowed down and eventually came to a halt. The reason was that an increasing number of highly similar microorganisms were (re)isolated, which led to subsequent rediscoveries of already known compounds.

> *"Given that only an extremely limited phylogenetic variety of microorganisms was studied in the past for their ability to produce antibiotics, our chances to find novel anti-infectives among the global microbial diversity are indeed high."*

A novel discovery strategy attempted to counteract this dearth of new discoveries by screening large synthetic compound libraries in conjunction with genome-based target identification, but the results did not live up to expectations (Tommasi *et al*, 2015). In fact, several publications from the pharmaceutical

1 Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research (HZI), Saarbrücken, Germany
2 Department of Pharmacy, Saarland University, Saarbrücken, Germany
3 Helmholtz International Lab for Anti-Infectives, Saarbrücken, Germany
*Corresponding author. E-mail: rolf.mueller@helmholtz-hips.de
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companies that pursued such strategies showed that it was relatively easy to find inhibitors of promising targets *in vitro*, but their translation into safe drugs—which are able to enter the bacterial cell to exert their effect—failed in almost every case.

What other options exist in light of these alarming developments? We should not discount the creativity and dedication of researchers around the globe, both in academia and in industry, to step up to such challenges. Indeed, there are several new approaches outside the scope of microbial natural products, including the development of small molecules and antibodies counteracting pathogenicity factors, strain-specific antibiotics, bacteriophages and vaccination, just to name a few. Nevertheless, most of these technologies either require companion antibiotics or still have not demonstrated clinical proof-of-concept.

Consequently, there is no reason not to go back to nature and search for novel antibiotics of microbial origin. Given that only an extremely limited phylogenetic variety of microorganisms was studied in the past for their ability to produce antibiotics, our chances to find novel anti-infectives among the global microbial diversity are indeed high. Estimates predict that < 1% of the global microbial diversity has so far been cultured (Locey & Lennon, 2016). Novel methods for exploring the remaining 99% for antibiotic discovery have continuously been developed during the past decades and are one reason for our cautious optimism that we can meet the challenge of antimicrobial resistance. This article gives a brief overview and critical comments on such approaches. The associated review article *Fighting antibiotic resistance—research strategies and (pre)clinical developments to find new antibacterials* gives an in-depth overview of antibiotic candidates currently under investigation for human use (See also: Walesch *et al*, 2022).

Shifting the focus onto previously neglected bacterial phyla

Soil-dwelling Actinomycetes, and especially the genus *Streptomyces*, which can be easily cultivated, are renowned for their ability to produce a large variety of antimicrobial compounds (Ahmed *et al*, 2020; Gavriilidou *et al*, 2022). Indeed, many antibiotics were originally isolated from these bacteria and Actinomycetes were used in screening campaigns by almost every pharmaceutical company during the 20th century. Yet, many compounds discovered by cultivation of these bacteria and screening of their culture extracts were already known, which has led to the above-mentioned increased rate of rediscoveries.

It is therefore necessary to shift the focus onto neglected bacterial phyla (Fig 1), namely organisms that cannot be isolated as easily as *Streptomyces*, but where researchers can develop methods for their isolation and cultivation from soil and other environmental samples. Researchers have barely scratched the surface of the biosynthetic potential of these neglected bacterial phyla and there is an increased chance for identifying novel kinds of (bio)chemistry there.

However, not every phylum harbors the same potential for natural product biosynthesis as Actinomycetes. Although genome analyses can help to identify genera which contain large numbers of biosynthetic gene clusters (BGC) (Gavriilidou *et al*, 2022), whole-genome sequences from a statistically significant number of representatives of each phylum are not yet available. Yet, it seems that bacteria with large genomes that occur ubiquitously or at least strive in diverse habitats show the largest potential for natural product biosynthesis. Furthermore, bacterial phyla only distantly related to *Streptomyces* might enable the discovery of BGCs that could yield novel types of antimicrobial secondary metabolites potentially targeting different bacterial mechanisms complementary to currently known antibiotics.

Examples for such neglected phyla include endosymbiotic bacteria, human commensals, other soil, but also marine bacteria. Our own work led us to specialize on *Myxobacteria*. These Gram-negative Deltaproteobacteria are ubiquitously present in soil and marine samples, show predatory behavior on various microorganisms, have the largest known bacterial genomes, and possess a superb potential for secondary metabolite production that rivals even *Streptomyces* (Garcia *et al*, 2010; Hoffmann *et al*, 2018; Panter *et al*, 2018; Gavriilidou *et al*, 2022).

Only few *Myxobacteria* strains have been sampled and studied because they are more difficult to cultivate and isolate than *Actinobacteria*. Through the development of a

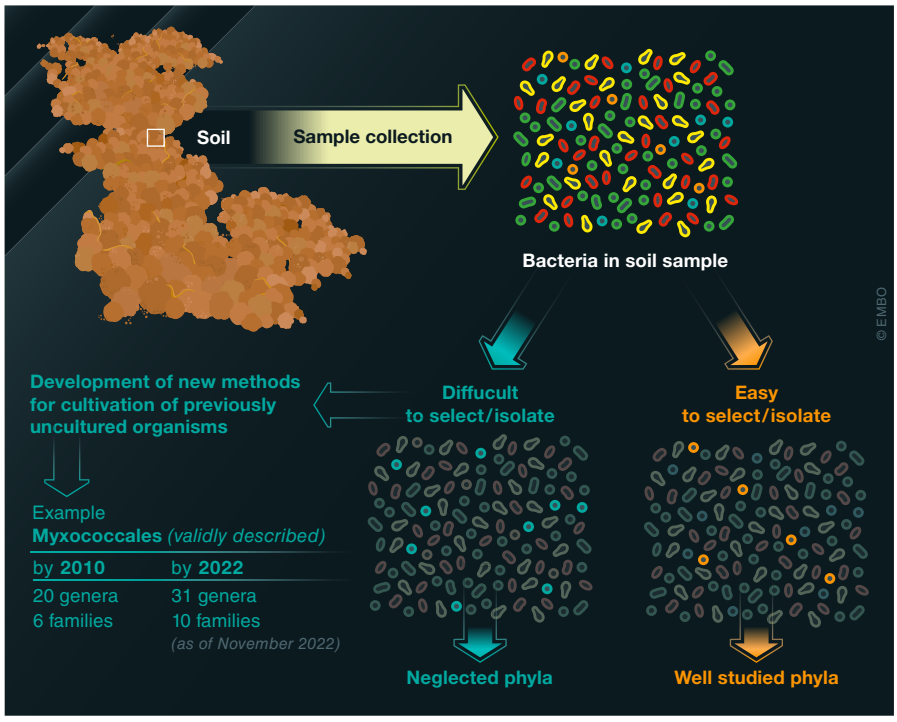

Figure 1. Soil samples can be a rich source of microbial diversity.
Bacteria easy to isolate are frequently studied, while bacteria that are more difficult to grow are less frequently sampled and thus often neglected by researchers.
Source data are available online for this figure.

robust methodology for isolation, our team at HIPS and HZI has now built an extensive library of *Myxobacteria* that is continuously being expanded. Importantly, we often isolate first members of novel genera, families, and even suborders that exhibit the highest potential for producing new kinds of natural products as shown in a comprehensive secondary metabolome study based on in-depth analysis of 2,300 *Myxobacteria* (Hoffmann *et al*, 2018). It is important to note that many of these isolates had been previously identified using metagenomic studies but were never cultivated. It is these "unculturable" strains that often bear the highest biosynthetic potential. The term "unculturable" is, however, debated among the scientific community, as these bacteria do strive in their natural habitats and hence it must be possible to define specific conditions for culturing them.

> *"... it seems that bacteria with large genomes that occur ubiquitously or at least strive in diverse habitats show the largest potential for natural product biosynthesis."*

The low sampling frequency for *Myxobacteria* and their ubiquitous presence also means that it is not necessary to travel to exotic places to make exciting new discoveries. In fact, we initiated a citizen scientist campaign to help us collect soil samples. The pilot project *Sample the Saarland* (https://hips.saarland/sample/?lang=en) was initially restricted to the German state of the Saarland, but we now have extended it to the whole of Germany and continuously add new sampling areas.

These efforts have been fruitful and led to the identification of novel lead structures, such as cystobactamid, a potent antimicrobial compound produced by *Cystobacter* sp. Cb v34 (Baumann *et al*, 2014). Our investments in microbiological expertise also led to a significant expansion of the cultured diversity of *Myxobacteria* from 20 genera and six families described in 2010 to currently 31 genera and 10 families as well as many more already isolated but not yet published (Garcia *et al*, 2010; Cao *et al*, 2019). We are convinced that similar approaches can be used to analyze and exploit other bacterial phyla.

Developing novel methodologies to cultivate previously uncultured organisms

While it is possible to cultivate some of the neglected bacterial phyla under laboratory conditions, there are many more species that are currently "unculturable": despite their abundance in nature, cultivation using traditional approaches has not been possible so far. Most estimations agree that the vast majority of bacteria falls into this category (Locey & Lennon, 2016). This collective of uncultured bacteria is often referred to as the "bacterial dark matter."

However, recent developments have shown that it is possible to tap into this source too. It requires innovative techniques and methods though that differ from traditional approaches by adding nutrients, changing growth conditions, or co-culturing with other species. One of the most prominent success stories in this regard is the discovery of the antimicrobial natural product teixobactin, which is produced by the Gram-negative bacteria *Eleftheria terrae* (Ling *et al*, 2015). The isolation of *E. terrae* was made possible with the so-called "isolation chip" (iChip) technology (Fig 2), which is basically a bacterial containment unit that is buried in the soil and allows the transfer of nutrients through a semipermeable membrane (Ling *et al*, 2015). The cells contained in the iChip have access to all the macro- and micronutrients in their native habitat, which circumvents the necessity to painstakingly identify essential nutrients for their cultivation.

Interestingly—and a bit counterintuitively—there seems to be a tendency among bacteria to be able to grow in simpler media after initial cultivation in the iChip. This was observed for the production of teixobactin after *E. terrae* was first isolated from the iChip (Ling *et al*, 2015). If this were not the case, it would not have been possible to grow this bacterium at large scale for compound isolation. Although there is no satisfying explanation for this behavior yet, the most likely one is that the cultivation of new strains under laboratory conditions cannot start from a few individual cells but requires a larger initial biomass. However, it cannot be ruled out that bacteria somehow adapt to a simpler environment during the initial cultivation in the iChip.

Following this lead, new technologies could be developed for other habitats to allow the sampling of so far unstudied organisms and their natural products for novel antimicrobial lead structures. A habitat that could be of particular interest in this context is the human microbiome, which contains many beneficial commensal bacteria co-evolving with their human hosts. These human commensals are producing many secondary metabolites with protective or otherwise beneficial features.

An example is a natural product of the lanthipeptide class produced by *Blautia producta* BP_{SCSK} (Kim *et al*, 2019). This compound does not only exhibit good *in vitro* activity against vancomycin-resistant *Enterococcus faecium* (VRE) but also the producing strain itself was shown to provide resistance against VRE infestation when colonizing the gut (Kim *et al*, 2019). *Escherichia coli* Nissle 1917 is another well-known gut probiotic strain that can protect the microbiota against colonization from pathogenic Enterobacteriaceae (Sassone-Corsi *et al*, 2016). It was recently demonstrated that its protective effect is derived from two antimicrobial natural products, microcin M and microcin H47 (Sassone-Corsi *et al*, 2016). Indeed, as more and more researchers start looking at cultivatable commensals, novel compounds with intriguing biological properties are being discovered. Thus, gaining access to so far uncultured members of the human microbiota could be very fruitful.

> *"... there are many more species that are currently "unculturable": despite their abundance in nature, cultivation using traditional approaches has not been possible so far."*

Animal microbiomes also warrant more scrutiny. For example, the unique and interesting compounds isolated from bacterial symbionts of marine sponges suggest a large potential for discovering anti-bacterials. So far, such compounds are often isolated by harvesting and extracting the whole sponge, which is not a sustainable practice, especially if a compound has to be tested in clinical trials and needs to be produced at a significant scale. Direct access to the bacterial sponge symbionts would therefore provide many exciting opportunities for antibiotic discovery.

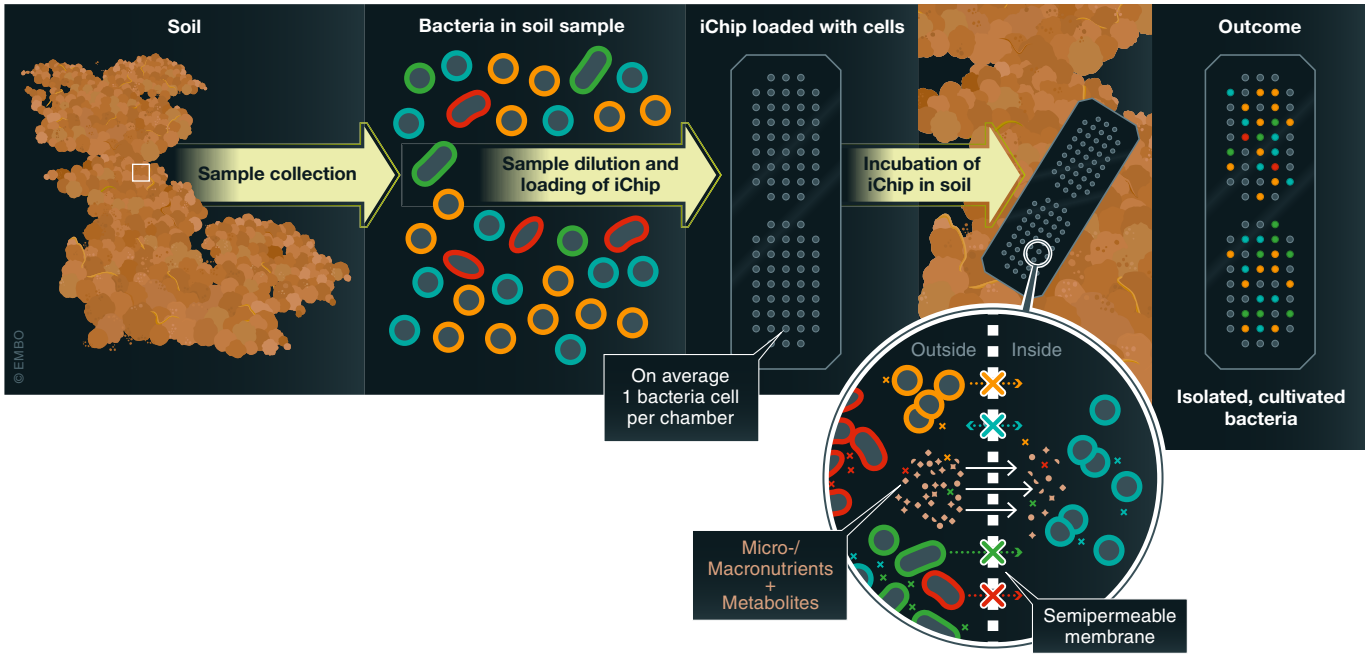

Figure 2. Simplified schematic representation of how the iChip technology can be utilized to cultivate bacteria from the soil that could not be isolated under standard laboratory conditions before.
Source data are available online for this figure.

Targeted genome mining of natural product biosynthetic gene clusters

The traditional identification and isolation of antimicrobial compounds from culture extracts is an untargeted approach with regard to the kind of natural products that will be identified. It is also limited to those natural products that the host strain synthesizes under cultivation conditions. The steady increase of sequenced genomes in public databases has shown that there is an untapped treasure trove of uncharacterized BGCs with an enormous potential to biosynthesize secondary metabolites. However, these are locked away in "cryptic biosynthetic gene clusters" that do not produce detectable amounts of compound under standard laboratory conditions.

Many research groups have therefore explored strategies to activate these clusters either directly in their native hosts or through transfer into a heterologous host. This important work takes considerable time and effort for discovering novel natural products, let alone isolating them in sufficient quantities for further study. One could basically regard this approach as a ladder to get to the higher branches on the tree of natural products as most of the low-hanging fruits have already been picked.

> *"... the unique and interesting compounds isolated from bacterial symbionts of marine sponges suggest a large potential for discovering antibacterials."*

While it takes longer to fill a basket this way, these efforts enable more selective targeting and isolation of new natural products from a specific compound class. As this approach is based on the prospecting of genomic databases to search for natural product BGCs, it is often referred to as "genome mining" (Fig 3). It starts with the identification of an unknown gene cluster of interest among the plethora of BGCs in databases. Researchers can then try to access the compounds predicted via genome mining by different means. One simple option would be to screen a manifold of culture media and alter cultivation conditions in the hopes of finding the right one under which the cells produce the natural product. Such screens can also include co-cultivation with other microorganisms—sometimes separated by semipermeable membranes—to provide environmental trigger signals that might switch on BGCs.

Another option is homologous or heterologous expression of BGCs identified by genome mining. This requires either genetic tools for manipulating the natural host (homologous expression) or appropriate host cells for heterologous expression. While *E. coli* is a very versatile and effective expression host for single proteins and small subsets of biosynthetic genes, it is often not a good choice for expressing multi-modular and complex mega-synthetases that are frequently involved in natural product biosynthesis. The likelihood of success is often tied to the phylogenetic relationship between the original bacterium and the host strain. Genome mining strategies have therefore greatly benefited from the development of new expression hosts or the improvement of existing ones. For example, the Luzhetskyy research group optimized the strain *Streptomyces albus* J1074 to be used as an expression host for natural product BGCs by deleting 15 existing natural product BGCs to reduce the metabolic background and channel more energy into the heterologous expression of BGCs (Ahmed *et al*, 2020). Similar strategies have also been carried out

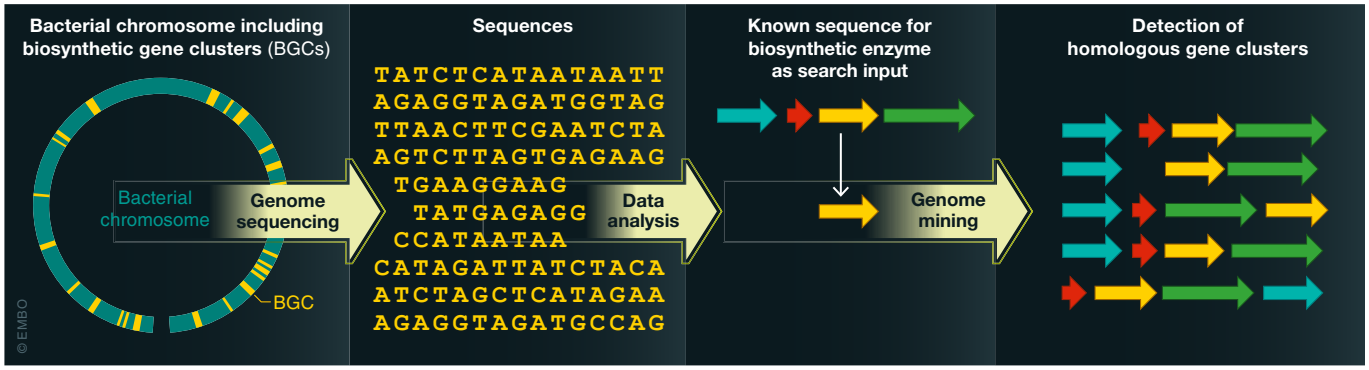

Figure 3. Schematic general workflow for genome mining for biosynthetic gene clusters homologous to a known cluster.
Source data are available online for this figure.

with other bacteria to turn them into expression hosts (Ahmed *et al*, 2020).

In addition to mining BGCs for unique and unknown products, the search for secondary metabolites closely related to already known ones enables the study of structure–activity relationships in an informed manner. By only sampling related structures that were conserved during evolution, we can make use of the fact that Nature has already preselected structure scaffolds to overcome existing antimicrobial resistance mechanisms. This search for natural analogues of active compounds led, for example, to the recent success story of the kanglemycins (Peek *et al*, 2018). Although these are structurally very similar to the clinically used bacterial RNA polymerase inhibitor rifampicin—itself a derivative of rifamycin-type natural products—the kanglemycins exhibit a potent activity against rifampicin-resistant bacteria.

Instead of searching for BGCs based on mere homology of the biosynthetic enzymes, another strategy is to mine for specific resistance-conferring proteins (Panter *et al*, 2018). If such enzymes are encoded in previously unknown natural product BGCs, it improves the chance that the metabolite produced by this BGC might exhibit an antibacterial activity, while the resistance gene provides self-immunity to the producing organism.

Sampling of environmental DNA and analysis of the resulting metagenomic data sets

Despite the aforementioned efforts to make additional species of bacteria cultivatable, these developments are currently only happening one at a time and not on a large scale. Thus, we are still left with a large quantity of bacteria, which we cannot study in a laboratory in the foreseeable future. However, there is still a way to glimpse into the natural product diversity of the bacterial dark matter by sampling and sequencing environmental DNA, that is, the entirety of DNA present in a sample taken from the environment (Fig 4). Combining the metagenomic data obtained this way with genome mining and heterologous expression (see above) facilitates the production and study of novel natural products originating from organisms not directly accessible to us (Gavriilidou *et al*, 2022). A manifold of sources can be used for generating metagenomic data sets, and samples taken from the soil or the ocean might be just as promising as samples from humans and animals (Gavriilidou *et al*, 2022).

Comparisons of metagenomic data sets from healthy individuals with the ones from patients with specific diseases might reveal changes in the microbiota associated with a disease state (Sassone-Corsi *et al*, 2016; Kim *et al*, 2019). Natural product BGCs that are present in the metagenomes of healthy individuals and absent in the patient might provide promising new chemical matter to be developed into drug leads.

The combination of environmental DNA sequencing to generate metagenomic data sets, genome mining, and heterologous overexpression is nowadays seen as the future standard route to get access to the natural product diversity in the bacterial dark matter. This approach obviously does not render efforts to make more microorganisms cultivatable obsolete, but it nonetheless complements them. Nevertheless, major hurdles need to be overcome: It is still difficult to assemble whole genomes from complex mixtures and consequently BGCs are often fragmented. Moreover, heterologous expression to test novel BGCs needs suitable chassis organisms capable of producing all required enzyme complexes, precursors, and self-resistance factors.

Concluding remarks

The threat of the ongoing antibiotic resistance crisis is becoming more prescient each day (Antimicrobial Resistance Collaborators, 2022), and the scientific community is working tirelessly on novel solutions. Research in academia in particular is pioneering new approaches to discover and develop new antibiotics for the fight of infectious diseases caused by resistant bacteria. It becomes apparent that the approaches we discussed here are mainly driven by recognizing the global microbial diversity as an evolutionary optimized resource for novel anti-infectives; the challenge is to gain access to their untapped chemical potential.

The hurdles to overcome in 2022 are indeed higher than in the mid-20th century during the golden age of antibiotic discovery, but there are nonetheless promising strategies. Moving away from the tried-and-true natural product producers of the past and studying so far neglected bacterial phyla will hopefully yield new lead structures inhibiting novel targets, thus making it more difficult for pathogens to develop resistance mechanisms. In addition, new strategies are being developed that enable the cultivation of novel types of bacteria that have never been studied in a laboratory before.

High-throughput sequencing techniques have become so economical and efficient that we can now sequence the genomes of

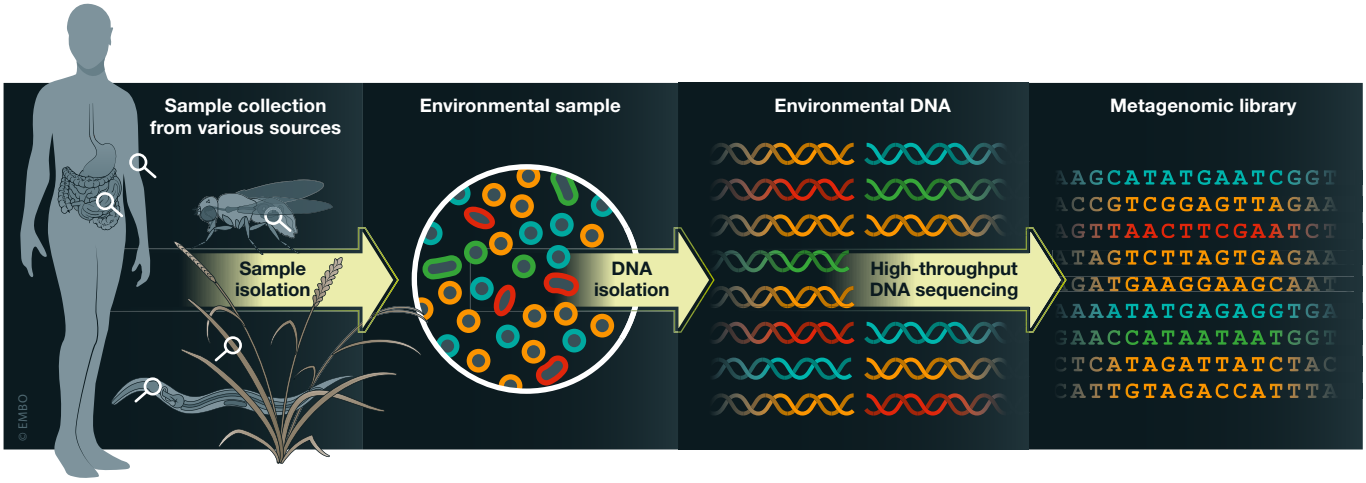

Figure 4. Schematic of the general workflow leading from the initial sample collection from the environment to the generation of a metagenomic library.
Source data are available online for this figure.

single cells found for example in a soil sample or a skin swab. In turn, research groups around the globe are developing new heterologous host strains to overexpress novel BGCs identified, which significantly increases the likelihood of finding novel natural products.

"Providing researchers with sufficient funds will accelerate and expand screening campaigns and thereby crucially improve the likelihood of antibiotic discovery."

Yet, despite the promises these novel strategies hold, the time needed for the development and clinical approval of new antibiotics is tightly tied to the available funding. Providing researchers with sufficient funds will accelerate and expand screening campaigns and thereby crucially improve the likelihood of antibiotic discovery. Making funding available to more risky and more laborious approaches will yield more innovation, which is direly needed in this field. A closer collaboration of academic research groups with industry might further boost novel developments and innovations as well. Both open discussion of such ideas as well as sensitizing the general populace for the problems ahead are important. The global COVID-19 pandemic has demonstrated the implications of infectious diseases lacking available treatment options in a painful and clear manner; maybe now would be a good time to start a public debate on antibiotic discovery.

In conclusion, we think that natural product research will remain crucial for resolving the looming antibiotic resistance crisis and we are looking forward to the creative new approaches and innovations coming from research groups all over the world. Even if we do not have a new golden age in the realm of antibiotic discovery at hand, we hope that the collaborate and joint efforts of academia and industry will be sufficient to prevent the worst.

Expanded View for this article is available online.

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References

Ahmed Y, Rebets Y, Estévez MR, Zapp J, Myronovskyi M, Luzhetskyy A (2020) Engineering of *Streptomyces lividans* for heterologous expression of secondary metabolite gene clusters. *Microb Cell Fact* 19: 5

Antimicrobial Resistance Collaborators (2022) Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* 399: 629–655

Baumann S, Herrmann J, Raju R, Steinmetz H, Mohr KI, Hüttel S, Harmrolfs K, Stadler M, Müller R (2014) Cystobactamids: myxobacterial topoisomerase inhibitors exhibiting potent antibacterial activity. *Angew Chem Int Ed* 53: 14605–14609

Cao P, Wei X, Awal RP, Müller R, Wall D (2019) A highly polymorphic receptor governs many distinct self-recognition types within the *Myxococcales* order. *MBio* 10: e02751-18

Garcia R, Gerth K, Stadler M, Dogma IJ Jr, Müller R (2010) Expanded phylogeny of myxobacteria and evidence for cultivation of the 'unculturables'. *Mol Phylogenet Evol* 57: 878–887

Gavriilidou A, Kautsar SA, Zaburannyi N, Krug D, Müller R, Medema MH, Ziemert N (2022) Compendium of specialized metabolite biosynthetic diversity encoded in bacterial genomes. *Nat Microbiol* 7: 726–735

Hoffmann T, Krug D, Bozkurt N, Duddela S, Jansen R, Garcia R, Gerth K, Steinmetz H, Müller R (2018) Correlating chemical diversity with taxonomic distance for discovery of natural products in myxobacteria. *Nat Commun* 9: 803

Kim SG, Becattini S, Moody TU, Shliaha PV, Littmann ER, Seok R, Gjonbalaj M, Eaton V, Fontana E, Amoretti L *et al* (2019) Microbiota-derived lantibiotic restores resistance against vancomycin-resistant *Enterococcus*. *Nature* 572: 665–669

Ling LL, Schneider T, Peoples AJ, Spoering AL, Engels I, Conlon BP, Mueller A, Schäberle TF, Hughes DE, Epstein S *et al* (2015) A new antibiotic kills pathogens without detectable resistance. *Nature* 517: 455–459

Locey KJ, Lennon JT (2016) Scaling laws predict global microbial diversity. *Proc Natl Acad Sci USA* 113: 5970–5975

Newman DJ, Cragg GM (2020) Natural products as sources of new drugs over the nearly four

decades from 01/1981 to 09/2019. *J Nat Prod* 83: 770 – 803

Panter F, Krug D, Baumann S, Müller R (2018) Self-resistance guided genome mining uncovers new topoisomerase inhibitors from myxobacteria. *Chem Sci* 9: 4898 – 4908

Peek J, Lilic M, Montiel D, Milshteyn A, Woodworth I, Biggins JB, Ternei MA, Calle PY, Danziger M, Warrier T *et al* (2018) Rifamycin congeners kanglemycins are active against rifampicin-resistant bacteria via a distinct mechanism. *Nat Commun* 9: 4147

Sassone-Corsi M, Nuccio SP, Liu H, Hernandez D, Vu CT, Takahashi AA, Edwards RA, Raffatellu M (2016) Microcins mediate competition among Enterobacteriaceae in the inflamed gut. *Nature* 540: 280 – 283

Tommasi R, Brown DG, Walkup GK, Manchester JI, Miller AA (2015) ESKAPEing the labyrinth of antibacterial discovery. *Nat Rev Drug Discov* 14: 529 – 542

Walesch S, Birkelbach J, Jézéquel G, Haeckl FPJ, Hegemann JD, Hesterkamp T, Hirsch AKH, Hammann P, Müller R (2022) Fighting antibiotic resistance—strategies and (pre)clinical developments to find new antibacterials. *EMBO Rep* https://doi.org/10.15252/embr.202256033