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Antibiotics from rare actinomycetes, beyond the genus *Streptomyces*

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Throughout the golden age of antibiotic discovery, *Streptomyces* have been unsurpassed for their ability to produce bioactive metabolites. Yet, this success has been hampered by rediscovery. As we enter a new stage of biodiscovery, omics data and existing scientific repositories can enable informed choices on the biodiversity that may yield novel antibiotics. Here, we focus on the chemical potential of rare actinomycetes, defined as bacteria within the order Actinomycetales, but not belonging to the genus *Streptomyces*. They are named as such due to their less-frequent isolation under standard laboratory practices, yet there is increasing evidence to suggest these biologically diverse genera harbour considerable biosynthetic and chemical diversity. In this review, we focus on examples of successful isolation and genera that have been the focus of more concentrated biodiscovery efforts, we survey the representation of rare actinomycete taxa, compared with *Streptomyces*, across natural product data repositories in addition to its biosynthetic potential. This is followed by an overview of clinically useful drugs produced by rare actinomycetes and considerations for future biodiscovery efforts. There is much to learn about these underexplored taxa, and mounting evidence suggests that they are a fruitful avenue for the discovery of novel antimicrobials.

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Introduction

The phylum Actinomycetota, formerly known as Actinobacteria [66], includes unicellular and filamentous bacteria that are commonly referred to as actinomycetes. The former includes human pathogens such as *Mycobacterium tuberculosis* (Mtb) and the industrial amino acid producer *Corynebacterium glutamicum*, whereas filamentous actinomycetes are studied for their complex developmental life cycles and their production of specialised metabolites. The type genus for this phylum is *Streptomyces*, which belongs to the filamentous group and includes more than 1100 verified species, producing specialised metabolites that form the basis of ~50% of clinically used antibiotics [42]. Their specialised metabolites are also used as cancer therapeutics (doxorubicin, daunorubicin), antifungal compounds (amphotericin, nystatin), immunosuppressants (rapamycin, FK506) and antiparasitic agents (ivermectins). The genes encoding the biosynthesis of these molecules are typically grouped together in biosynthetic gene clusters (BGCs) alongside genes involved in immunity, regulation and transportation. As discussed below, a recent survey of bacterial (meta)genomes revealed that only three

percent of their BGCs have been matched to metabolites, suggesting there are many more useful pathways and chemical products waiting to be discovered [34].

'Rare' actinomycetes

The term 'rare' actinomycetes, is an artificial grouping of all actinomycete genera, except for *Streptomyces*. The taxa included in this grouping are often ambiguous. Most view this as strains within the class Actinomycetes (with varying agreement on whether this is only filamentous strains or not), with the genus *Streptomyces* excluded. To provide a comprehensive overview, we define this as *all* genera within the Actinomycetes, except *Streptomyces*. These include, but are not limited to, *Actinoallomurus*, *Actinoalloteichus*, *Actinocorallia*, *Actinokineospora*, *Actinomadura*, *Actinopolyspora*, *Actinoplanes*, *Actinospica*, *Actinosynnema*, *Aeromicrobium*, *Agromyces*, *Alloactinosynnema*, *Allokutzneria*, *Amycolatopsis*, *Beutenbergia*, *Catellatospora*, *Catenulispora*, *Catenuloplanes*, *Cellulosimicrobium*, *Corynebacterium*, *Couchioplanes*, *Dactylosporangium*, *Dietzia*, *Frankia*, *Gordonia*, *Isopterocola*, *Jiangella*, *Knoellia*, *Kocuria*, *Krasnikoviella*, *Kribbella*, *Kutzneria*, *Lentzea*, *Microbacterium*, *Microbispora*, *Micromonospora*, *Mumia*, *Mycobacterium*, *Nonomuraea*, *Nocardia*, *Nocardioides*, *Nocardiopsis*, *Oerskovia*, *Planobispora*, *Planomonospora*, *Plantactinospora*, *Pseudokineococcus*, *Pseudonocardia*, *Rhodococcus*, *Saccharomonospora*, *Saccharopolyspora*, *Saccharothrix*, *Salinospora*, *Streptosporangium*, *Thermobispora* and *Tsakamurella*.

Contrary to popular belief, this naming is not a reflection of their abundance in nature, but because they are less frequently isolated and relatively understudied compared with the genus *Streptomyces*. This is likely due to their slower growth (in laboratory environments) and the lack of targeted isolation methods. Increasing evidence suggests that the 'rare' should be dropped, because explorations of, for example, *Amycolatopsis*, *Micromonospora*, *Pseudonocardia*, *Saccharopolyspora* and *Salinospora*, have yielded chemically diverse and novel specialised metabolites. The potential of these genera has been the focus of recent review articles [2,25,50], encompassing strains from diverse environments [30,87] or focussing on a particular genus, such as *Micromonospora* [41]. As such, in the quest for new antimicrobial agents, it may be advantageous to not rely on over-sampled taxa, such as *Streptomyces*, but instead expand our knowledge, resources and expertise across these under-represented genera.

A recent survey of microbial genomes reported that *Streptomyces* encodes the greatest chemical diversity within the Actinomycetes, but that *Amycolatopsis* and *Micromonospora* also have significant biosynthetic potential [34]. As shown in Figure 1, the number of sequenced *Streptomyces* strains far exceeds all other

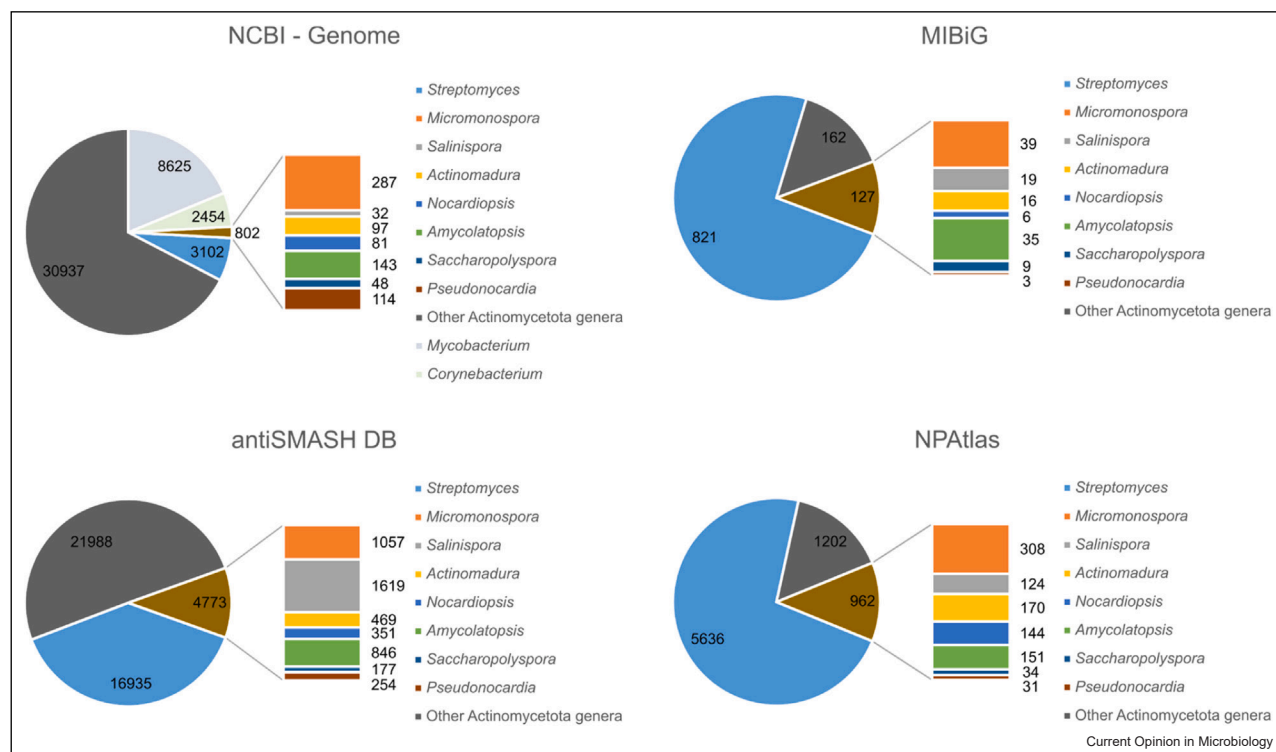
filamentous actinomycete genera, with the next most common being *Amycolatopsis* and *Micromonospora*, this is discussed further below. Other genera include *Pseudonocardia* that is well-studied largely because of its mutualistic symbiosis with fungus-farming ants in the tribe *Attini*, which are endemic to South and Central America [7]. The queen of an ant colony vertically transmits a single strain of mutualist *Pseudonocardia* to worker ants, who cultivate the bacterium on their cuticles, providing it with food and shelter in return for antibiotics; these antibiotics influence the ant microbiome and provide a means to protect themselves and their food supply from *Escovopsis*, a genus of parasitic fungi [101]. Other filamentous actinomycetes have also been isolated from these ants, including *Amycolatopsis* and *Streptomyces* [49,78,81,101], but the stable association is typically with *Pseudonocardia* species [15]. Additionally, species of *Saccharopolyspora* and *Streptomyces* have been isolated from fungus-growing ants in Kenya and have yielded novel antibiotics [73,79].

Evidence is accumulating that free-living soil actinomycetes have also adapted to colonise plants and animals, as observed in the well-studied *Pseudonocardia*-attine ant, *Streptomyces*-beewolf, and *Streptomyces*-bark beetle symbioses [80,93]. Rare actinomycetes have been isolated from diverse environments, including soil, freshwater, marine animals and deep-sea sediments, and from the roots of plants and trees. In terrestrial ecosystems, plant root exudates may provide nutrients, so it is perhaps unsurprising that many species of *Pseudonocardia* [74], *Micromonospora* [104], *Saccharopolyspora* [76] and *Streptomyces* have been isolated from plant roots. In some cases, they have been shown to be highly enriched in the rhizosphere and endosphere of plant roots [71].

The marine environment has also been shown to be a rich source of rare actinomycetes [50]. In fact, many of the 31 verified *Saccharopolyspora* species were isolated from marine environments and 16 have been described as halotolerant or halophilic [76]. The obligate marine genus *Salinispora* is perhaps one of the most comprehensively studied for their biosynthetic and chemical potential and has been proposed as a model organism for specialised metabolite discovery [44]. The potent proteasome inhibitor, salinosporamide A [32], produced by *Salinispora tropica* [43], is currently in phase-three clinical trials as an anticancer drug [8]. Aside from the structurally diverse and novel chemistry produced by this genus, a culture collection of thousands of strains [75] has enabled a comprehensive assessment of the diversity and evolution of specialised metabolism across the *Salinispora* genus [28,51,108].

Despite the success stories from the genera studied in detail, many remain vastly understudied, this includes

Figure 1



Number of entries per genus in the National Center for Biotechnology Information (NCBI) Genome, antiSMASH, MIBiG and NPAtlas databases for the phylum Actinomycetota.

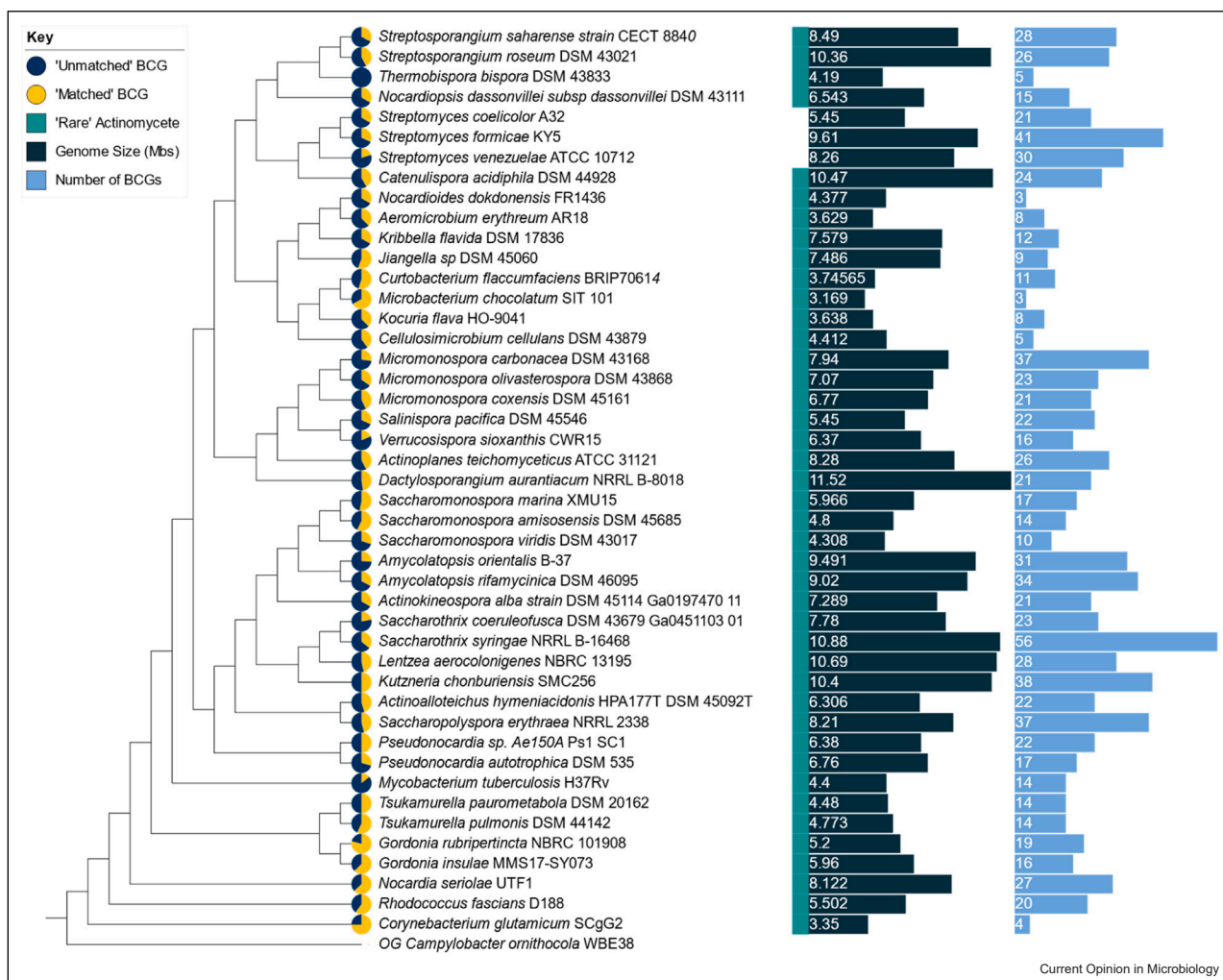
Catellatospora, of which three new plant-growth-promoting ansamacrolactams have recently been discovered [54] and *Saccharothrix*, where strains from the marine environment were found to produce eight novel, cytotoxic amphiphilic siderophores [84]. Genome sequencing and mining of existing genomic databases combined with availability through culture collections can certainly aid the prioritisation of strains.

Chemical and biosynthetic potential

As noted above, it has been estimated that only three percent of bacterial BGCs have been matched to known products [34]. Among bacteria, the Actinomycetota phylum harbours a particularly high biosynthetic potential, dedicating up to 3.0 Mb of coding capacity, over 15% of their genome, to specialised metabolite biosynthesis [3,18]. Despite the biosynthetic potential of this phylum, fewer than 10% of the sequenced microbial genomes available in public repositories belong to this group [45]. Furthermore, it has been estimated that currently only 30–50% of the biological diversity of this phylum is represented by sequenced strains [83]. The potential of the *Streptomyces* genus, as mentioned previously, is reflected in the high number of BGCs with their genomes [46]. In fact, a comparison of biosynthetic

diversity calculated as unique gene cluster families showed that the *Streptomyces* genus is responsible for most of the phyla-level biosynthetic diversity [34]. Another comparative analysis based on the abundance of BGCs per genome, identified the orders Pseudonocardiales, Streptosporangiales and Micromonosporales as gifted taxa, with an average of 19.8, 15.0 and 13.3 BGCs per genome, respectively, in comparison with the 21.6 calculated for the order Streptomycetales [27]. However, although rare actinomycetes usually have fewer BGCs per genome, their novelty and diversity could be higher than *Streptomyces*. For example, a study performed with marine-derived actinomycetes showed that when gene diversity was normalised by the number of BGCs, it was higher in rare actinomycetes, with the genera *Corynebacterium*, *Gordonia*, *Nocardioopsis*, *Saccharomonospora* and *Pseudonocardia* representing the highest BGC diversity [77]. This study also determined that phylogeny and genome size combined can be used to predict the likelihood of BGC diversity. Similarly, recent work that applied a genomic metric called biosynthetic novelty index (BiNI) to assess BGC novelty was applied to actinomycetes strains from various taxonomic and ecological backgrounds and revealed that *Streptosporangium*, *Lentzea*, *Actinokineospora* and *Saccharothrix* genera exhibited the highest novelty index [35]. Similarly, a

Figure 2



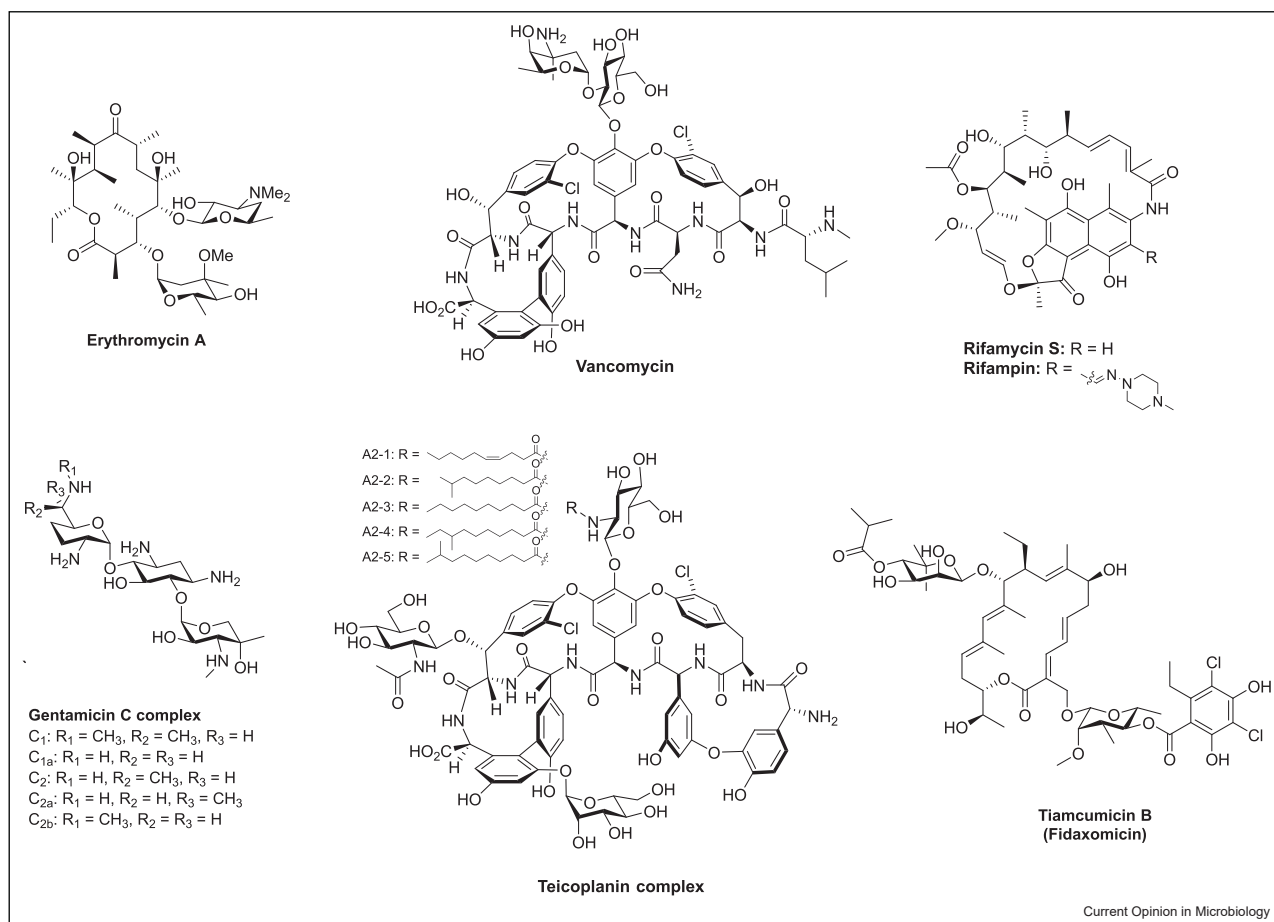
Phylogenetic tree generated by AutoMLST [1] showing genome size, number of BCGs predicted by antiSMASH and the proportion of these that are predicted to be more than 10% of a match to previously characterised clusters identified by antiSMASH (in the MIBiG database). The input to AutoMLST was 20 representative, complete genomes of genera discussed in this review.

Nonomuraea sp. was recently found to harbour the largest actinomycete chromosome encoding approximately 32 BCGs [65]. However, not all rare actinomycetes appear to be as talented. For example, genome sequencing of genera such as *Micrococcus* and *Agrococcus* demonstrated less than five predicted BCGs per genome [86], and whilst this does not preclude these metabolites from being useful, it does highlight the incredible variation within this group.

The frequent rediscovery of known specialised metabolites typically comes from screening multiple isolates of common *Streptomyces* strains [96]. Representatives of the Actinomycetota, and their numbers of predicted BGC are shown in Figure 1, with *Micromonospora*, *Salinispora*, *Actinomadura*, *Nocardioopsis*, *Amycolatopsis*,

Saccharopolyspora and *Pseudonocardia* identified as common 'rare actinomycete' genera across the three databases (Figure 1). The antiSMASH database (antiSMASH DB), which provides pre-calculated antiSMASH annotations for completed, publicly available bacterial genomes [10,11], shows that 38% of Actinomycetota BCGs belong to the *Streptomyces* genus (Figure 2). The prevalence of *Streptomyces*-encoded specialised metabolites increases when manually curated metadata, such as specialised metabolite chemical identity, are included. In the Minimum Information about a Biosynthetic Gene cluster (MIBiG) database [88], 74% of Actinomycetota BCGs belong to the *Streptomyces* genus. A similar situation is observed for the Natural Products Atlas (NPAtlas), a database of known microbial-specialised metabolite structures [94], where 72% of

Figure 3



Chemical structures of antibacterial drugs derived from natural products of rare actinomycetes. Rifampicin is a semisynthetic molecule derived from rifamycin S, and the active pharmaceutical ingredient (API) of gentamicin and teicoplanin comprises a complex mixture of congeners, the most abundant of which are shown in each case.

specialised metabolites were isolated from *Streptomyces* strains. As such, even though antiSMASH DB contains a considerable number of rare actinomycete BGCs, only a very small proportion of their products has been chemically characterised (i.e. correlated MIBiG and NPAtlas database results).

Of the 45,956 Actinomycetota genomes in the NCBI database, 3234 are complete genome sequences and only 630 of these are NCBI reference sequences of rare actinomycetes [72]. To highlight the biosynthetic potential of rare actinomycetes, reference genomes from diverse genera were selected, including where possible, those mentioned in this review. The abundance and phylogenetic distribution of (un)characterised BGCs harboured by rare actinomycetes is shown in Figure 2. This analysis reinforces common knowledge, that is, that *Streptomyces* sp. typically harbour more BGCs that have already been characterised or are highly matched to those that have, further illustrating a lower potential for

novel discoveries. For example, *S. venezuelae* and *S. coelicolor* showed 24/30 and 14/21 ‘matched’ BGCs, respectively. In contrast, the rare actinomycete *Saccarothrix syringae* shows a remarkable number of predicted BGCs. AntiSMASH predicted that the 10.88-mb genome contained 56 BGCs, of which 20 of these had 10% or less similarity to known BGCs. In addition, although there are strains with a lower number of predicted BGCs, for example, *Gordonia insulae* only encodes 16 predicted BGCs in its 5.96-Mbp complete genome, 10 of them are predicted to be novel BGCs. Whilst these types of analyses have caveats, including the assumption of accurate assembly, large-scale genome mining data have confirmed that there is extensive novel biosynthetic potential encoded in rare actinomycete genomes.

Clinically used drugs from rare actinomycetes

In total, five classes of clinically used antibacterial drugs are derived from rare actinomycetes, several of which are

Table 1
Examples of clinically used drugs from rare actinomycetes.

Drug	Producing organism	Class	Mode of action	Discovered (approved)
Erythromycin [59]	<i>Saccharopolyspora erythraea</i>	Macrolide (14-atom ring)	Binds ribosome 50S subunit to inhibit protein synthesis	1952 (1952)
Vancomycin [58]	<i>Amycolatopsis orientalis</i>	Glycopeptide	Binds lipid II and inhibits cell wall synthesis	1954 (1958)
Teicoplanin [67]	<i>Actinoplanes teichomyceticus</i>	Glycopeptide	Binds lipid II and inhibits cell wall synthesis	1978 (1984)
Rifamycin [82]	<i>Amycolatopsis rifamycinica</i>	Ansamycin	Inhibits bacterial RNA polymerase to block transcription	1959 (1963)
Gentamicin [98]	<i>Micromonospora purpurea</i>	Aminoglycoside	Binds ribosome 30S subunit to inhibit protein synthesis	1963 (1964)
Fidaxomicin [68]	<i>Dactylosporangium aurantiacum</i> subsp. <i>hamdenesis</i>	Macrolide (18-atom ring)	Inhibits open-complex formation by bacterial RNA polymerase	1975 (2011)

on the WHO list of Essential Medicines [99] (Figure 3, Table 1). The oldest of these is the macrolide antibiotic erythromycin produced by *Saccharopolyspora erythraea*, introduced into clinical use over 70 years ago [59]. Macrolide antibiotics, with a 14-atom ring size, inhibit protein synthesis and are particularly useful for the treatment of upper respiratory tract infections. Yet, erythromycin suffers from acid instability that is linked to its common gastrointestinal side effects and action as a motilin agonist [97]. These and other limitations are overcome to some degree by a range of semisynthetic variants of which clarithromycin and azithromycin are the most successful [26]. This motilin agonist activity means erythromycin is commonly used off-label for gastroparesis, and its immunomodulatory activity has led to low-dose off-label use in Southeast Asia for the treatment of diffuse panbronchiolitis [53]. Intriguingly, nature has produced an acid-stable erythromycin congener called sporeamicin, which is also the product of a *Saccharopolyspora* species [103], but the biosynthetic steps that differentiate these pathways remain unresolved.

The glycopeptide vancomycin produced by *Amycolatopsis orientalis* [58] inhibits cell wall biosynthesis by binding to the lipid-II precursor. This is prescribed for life-threatening infections by Gram-positive bacteria that are resistant to other treatments: this reserved use stems, in part, from a range of serious side effects. Glycopeptides are poorly absorbed by the gastrointestinal tract, meaning they are mostly restricted to intravenous use, except for *Clostridioides difficile* infection (CDI) treatment. The related glycopeptide teicoplanin, produced by *Actinoplanes teichomyceticus*, differs most notably in two of the seven amino acids that form its backbone, and in the glycosylation pattern [16,67]. Semisynthetic modification of these and the related A40926 family of glycopeptides, produced by an *Actinonmadura* sp., led to the development of the second-generation lipoglycopeptide drugs telavancin, oritavancin and dalbavancin [9].

Rifamycin, first isolated from *Amycolatopsis rifamycinica* in 1959, is the founding member of the ansamycin family of polyketides [82]. It is an inhibitor of the DNA-dependent RNA polymerase, but the metabolite itself lacks the properties required for effective pharmacological application, meaning semisynthetic modification was required to produce successful drug molecules [33]. The most widely used is rifampin that was first marketed in 1968 and has become a first-line antituberculosis therapy when used in combination with other agents. The rifamycin congener kanglemycin A produced by *Amycolatopsis* spp. maintains potency against RNA polymerases containing rifampicin-resistant mutations and has become the subject of significant recent interest as a lead for semisynthetic optimisation [61,69].

The aminoglycoside antibiotic gentamicin is produced by *Micromonospora purpurea* and was introduced into clinical use in 1964 [98]. Aminoglycosides can be highly effective for the treatment of Gram-negative pathogen infections, in particular sepsis, and target the bacterial ribosome 30S subunit, inhibiting protein synthesis by interfering with initiation, codon fidelity and translocation. However, their use is often limited due to side effects, and gentamicin displays significant renal and ototoxicity. The pharmaceutical formulation gentamicin C comprises a complex of five congeners and there is evidence to suggest that individual components may have lower toxicity, which has led to efforts for pathway engineering [48]. The mechanism of action, which interferes with codon fidelity, means gentamicin is being repurposed for the treatment of genetic diseases such as Duchenne muscular dystrophy in which mutations lead to the presence of premature stop codons leading to truncated proteins [95].

The most recently approved antibacterial drug (2011) to come from a rare actinomycete is fidaxomicin for CDI, an inhibitor of RNA polymerase that inhibits the same enzyme but acts through a different mechanism to rifamycin-based molecules [29]. The active entity of fidaxomicin is the 18-membered macrolide tiacumicin produced by *Dactylosporangium aurantiacum* subsp. *hamedensis*. Tiacumicin B was first discovered in 1986 [89] and is part of a larger group of more than 40 related rare actinomycete-specialised metabolites, the first being lipiarmycin that was discovered in 1975 from *Actinoplanes deccanensis* [21,68]. This delay in development stems from poor oral bioavailability limiting broad-spectrum application, but this would later prove beneficial for the narrow-spectrum treatment of CDI and the resurrection of this compound class.

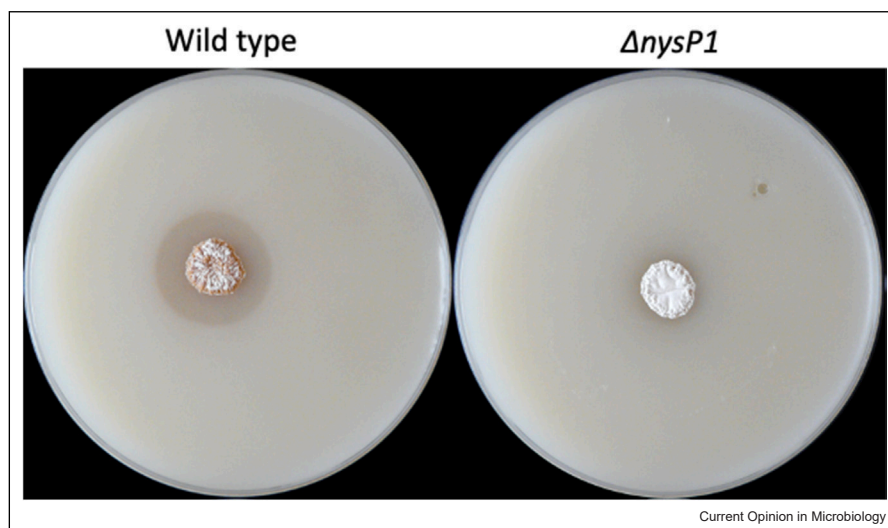
Beyond the approved drugs, peptide antibiotics produced by rare actinomycetes have proven very successful as leads for the treatment of CDI [70]. These include the glycolipodepsipeptide ramoplanin that is a cell wall-targeting antibiotic produced by *Actinoplanes ramoplaninifer* [23,56]. LFF-571 is a semisynthetic derivative of the thiopeptide GE2270A produced by *Planobispora rosea* that inhibits protein synthesis via bacterial elongation factor thermal unstable (EF-Tu) [62], and NVB302 is a semisynthetic analogue of the type-B lantipeptide deoxyactinogardine produced by *Actinoplanes liguriae* that binds the cell wall precursor lipid II [12]. All three peptides are narrow-spectrum agents for the treatment of CDI, but while ramoplanin is being fast-tracked through Phase-III clinical trials, the development of LFF-571 and NVB302 appears to have halted. Very recently, semisynthetic variants of the unusual macrolide sequanamycin produced by *Allokutzneria albata* were reported as candidates with utility against Mtb that overcomes the inherent macrolide resistance of Mtb [105].

Considerations for the future study of rare actinomycetes

Recent advances in genetic modification systems for actinomycetes have been the subject of several review papers [24,60], including genetic modification focussed on cloning and heterologous expression in *Streptomyces* species [64,102]. Protocols for genetic engineering of actinomycetes, as well as vector maps, are freely available on <http://actinobase.org> [23,31] where you can also download a free pdf copy of the Practical *Streptomyces* Genetics manual, first published in 1985, and updated in 2000 [47]. Genetic engineering has provided a promising avenue for exploiting the biosynthetic potential of microorganisms, but in the case of actinomycetes, most of the protocols were developed for *Streptomyces* species, and are not generally applicable to other genera without modifications [55]. For example, while site-specific recombination systems based on bacteriophage integrases have been a fundamental tool for *Streptomyces* genetic engineering, mediated by phage-based vectors such as pSET152 and pMS82, the application of these tools in rare actinomycetes depends largely on the presence of the same genetic elements [31,37]. Bacteriophage integration occurs between DNA sequences on the phage attachment site and the host genome on the bacterial attachment site (*attB*) [20]. While innate *attB* sites are regularly found in *Streptomyces* genomes, they are less conserved in rare actinomycetes [4], but an *attB* sequence in *Pseudonocardia alni*, with an identity of 89% to the canonical *attB* sequence in *S. coelicolor*, facilitated its genetic engineering to produce a host mutant ([52], p. 200). In many cases, the *attB* site could be also introduced before recombination and this strategy was successfully applied to produce a *Salinispora tropica* mutant [106,107]. The yeast meganuclease I-SceI has also been introduced into *Streptomyces* species on an integrative phage vector and used to make targeted double-strand breaks, but this is mediated by a self-replicating plasmid and such plasmids would need to be identified and/or developed for use in rare actinomycetes [85].

In the absence of tools to make precise gene deletions, it is sometimes possible to disrupt genes using a suicide vector approach, that is, by introducing a non-replicating vector through conjugation with *E. coli*. A suicide vector approach to disrupt the nystatin P1 antifungal BGC was successful in *Pseudonocardia* P1 [6]. The disruption plasmid harboured an ~1500-bp internal fragment of *nypI* polyketide synthase as well as an apramycin resistance cassette and conjugal origin of transfer, which was used to mobilise the plasmid from *E. coli* to *Pseudonocardia* P1. Integration of the disruption plasmid into *nypI* abolished the ability of *Pseudonocardia* P1 to inhibit *Candida albicans* in a whole-cell bioassay (Figure 4). Recent advances in Clustered Regularly Interspaced Short Palindromic Repeats-associated protein 9 (CRISPR-Cas9)-based

Figure 4



Bioassay plates showing colonies of wild-type *Pseudonocardia* P1 and an isogenic mutant in which a suicide vector was inserted into *nyp1* polyketide synthase gene responsible for production of the polyene antifungal nystatin P1. Both colonies are overlaid with soft agar inoculated with the human pathogen *Candida albicans*. Disruption of *nyp1* abolished the zone of inhibition, suggesting this strain no longer makes nystatin P1.

engineering also represent an opportunity to precisely edit the genomes of rare actinomycetes, spanning multiple genera [40,91]. For example, CRISPR–Cas9-based techniques have been successfully applied in *Actinoplanes* [100] and *Micromonospora* [13,19], but again this is dependent on successful conjugation and plasmid replication in these bacteria. If conjugation is not possible, then polyethylene glycol (PEG)-mediated protoplast transformation is another historically important tool in *Streptomyces* genetics that has successfully applied in rare actinomycetes. However, the efficiency for protoplast formation and regeneration varies widely depending on the strain and seems to be less effective than in *Streptomyces* species [63].

Despite evidence that vertical inheritance is critical for BGC diversity [17], it is widely accepted that novel habitats, lifestyles or ecological interactions of rare actinomycetes may influence their potential to harbor novel BGCs and thus in turn, influence the structural diversity of their produced metabolites. This functional diversification can result from both abiotic and biotic ecosystem pressures, and the latter includes drivers such as signalling, protection and defence. Even with these widely accepted views, studying chemical ecology linked to function is a challenge and there is limited evidence to pinpoint this to biosynthetic diversification. Studies showing the recruitment of BGCs as a strategy for ‘sampling from the environment’ before becoming more ‘fixed’ in a population are still in their infancy [14,108], yet they provide an exciting step forward in our understanding of ecology and BGC evolution.

In order to incorporate ecological thinking across taxa, we must first improve our ability to capture these less-studied species. Part of the challenge of working with rare actinomycetes, is they are harder to grow and often grow more slowly under standard culture conditions compared with *Streptomyces* strains. For example, in our experience, *Pseudonocardia* strains isolated from attine ants take 2–4 weeks to grow, compared with 2–4 days for many *Streptomyces* species, and *Pseudonocardia* bacteria can typically only be cultured on solid agar. In soil, *Streptomyces* species have been found to be the dominant group among the total actinomycete population, although of course this could be biased based on approach [5,36]. However, selective isolation of a particular rare actinomycete genus must involve techniques that enhance the growth of desirable actinomycetes (enrichment) and eliminate *Streptomyces* and other undesirable taxa from the isolation media [39,90]. There have been several relatively recent advances in the area of isolation and culturing of previously unculturable organisms. One example that was successful for Nanoarchaeota was fluorescent *in situ* hybridisation and fluorescence-activated cell sorting to specifically isolate live cells of interest, allowing them to be cultured [38]. High-throughput dilution to extinction experiments has been effective with marine organisms by avoiding the need for artificial media whilst removing other, potentially faster-growing microorganisms [38]. The availability of metagenomes also enables scientists to specifically target organisms of interest. For example, Cross and colleagues described a method termed ‘reverse genomics isolation’ in which cell surface proteins were predicted from

sequencing data and allowed for specific target antibodies to be generated [22]. Moving forward, a range of techniques, old and new, will be required along with communication of results. One solution is the sharing of methods and protocols through community platforms such as <http://actinobase.org> [31]. Another solution is to adapt a culturing approach that mimics their natural environment more closely, such as soil mesocosms and microcosms, as was demonstrated through the impact of bacterial community structure on the metabolome of marine sediments [92]. Another strategy to elicit new chemistry is that of co-culture because it is well-known that microorganisms exist in complex multi-species/kingdom niches and, as such, competition and interactions are commonplace. There are several excellent pioneering ecology studies focussed on such interactions, including mutualism and competition, for example, interactions of the genus *Couchioplanes* [57], and such approaches could be particularly useful if the goal is to elicit specialised metabolites with antibiotic potential.

In summary, rare actinomycetes are under-represented in natural product databases, they exhibit more BGC diversity than streptomycetes and their potential is often undervalued. While we have outlined some considerable experimental hurdles to working with these genera, including genetic tractability, isolation and metabolite elicitation, they harbour considerable biosynthetic potential and it is worthwhile developing tools to exploit these bacteria. Their taxonomic and ecological diversity is both a blessing and a curse and success will come from shared knowledge across strains and communities, and from perseverance. There are some taxonomic starting points for rare actinomycete natural product discovery, for example, from *Pseudonocardia*, *Salinispora* and *Micromonospora* species, and community data sharing will enable us to evaluate rare actinomycete genera and thus be informed about where efforts are best spent. In the future, these understudied taxa may be anything but rare.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships that may be considered as potential competing interests: K DUNCAN reports financial support was provided by Biotechnology and Biological Sciences Research Council.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
 - of outstanding interest.
1. Alanjary M, Steinke K, Ziemert N: **AutoMLST: an automated web server for generating multi-locus species trees highlighting natural product potential.** *Nucleic Acids Res* 2019, **47**:W276-W282, <https://doi.org/10.1093/nar/gkz282>
 2. Amin DH, Abdallah NA, Abolmaaty A, Tolba S, Wellington EMH: **Microbiological and molecular insights on rare Actinobacteria harboring bioactive prospective.** *Bull Natl Res Cent* 2020, **44**:5, <https://doi.org/10.1186/s42269-019-0266-8>
 3. Baltz RH: **Gifted microbes for genome mining and natural product discovery.** *J Ind Microbiol Biotechnol* 2017, **44**:573-588, <https://doi.org/10.1007/s10295-016-1815-x>
 4. Baltz RH: **Streptomyces temperate bacteriophage integration systems for stable genetic engineering of actinomycetes (and other organisms).** *J Ind Microbiol Biotechnol* 2012, **39**:661-672, <https://doi.org/10.1007/s10295-011-1069-6>
 5. Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Klenk H-P, Clément C, Ouhdouch Y, van Wezel GP: **Taxonomy, physiology, and natural products of Actinobacteria.** *Microbiol Mol Biol Rev* 2015, **80**:1-43, <https://doi.org/10.1128/MMBR.00019-15>
 6. Barke J, Seipke RF, Gruschow S, Heavens D, Drou N, Bibb MJ, Goss RJM, Yu DW, Hutchings MI: **A mixed community of actinomycetes produce multiple antibiotics for the fungus farming ant *Acromyrmex octospinosus*.** *BMC Biol* 2010, **8**:109, <https://doi.org/10.1186/1741-7007-8-109>
 7. Batey SFD, Greco C, Hutchings MI, Wilkinson B: **Chemical warfare between fungus-growing ants and their pathogens.** *Curr Opin Chem Biol, Mech Biol Energy* 2020, **59**:172-181, <https://doi.org/10.1016/j.cbpa.2020.08.001>
 8. Bauman KD, Shende VV, Chen PY-T, Trivella DBB, Gulder TAM, Vellalath S, Romo D, Moore BS: **Enzymatic assembly of the salinosporamide γ -lactam- β -lactone anticancer warhead.** *Nat Chem Biol* 2022, **18**:538-546, <https://doi.org/10.1038/s41589-022-00993-w>
 9. Blaskovich MAT, Hansford KA, Butler MS, Jia Z, Mark AE, Cooper MA: **Developments in glycopeptide antibiotics.** *ACS Infect Dis* 2018, **4**:715-735, <https://doi.org/10.1021/acsinfecdis.7b00258>
 10. Blin K, Shaw S, Kautsar SA, Medema MH, Weber T: **The antiSMASH database version 3: increased taxonomic coverage and new query features for modular enzymes.** *Nucleic Acids Res* 2021, **49**:D639-D643, <https://doi.org/10.1093/nar/gkaa978>
 11. Blin K, Shaw S, Kloosterman AM, Charlop-Powers Z, van Wezel GP, Medema MH, Weber T: **antiSMASH 6.0: improving cluster detection and comparison capabilities.** *Nucleic Acids Res* 2021, **49**:W29-W35, <https://doi.org/10.1093/nar/gkab335>

12. Boakes S, Dawson MJ: **Discovery and development of NVB302, a semisynthetic antibiotic for treatment of *Clostridium difficile* infection.** John Wiley & Sons, Ltd; 2014:455-468, <https://doi.org/10.1002/9781118794623.ch24>
13. Braesel J, Lee J-H, Arnould B, Murphy BT, Eustáquio AS: **Diazaquinomycin biosynthetic gene clusters from marine and freshwater actinomycetes.** *J Nat Prod* 2019, **82**:937-946, <https://doi.org/10.1021/acs.jnatprod.8b01028>
14. Bruns H, Crüsemann M, Letzel A-C, Alanjary M, McInerney JO, Jensen PR, Schulz S, Moore BS, Ziemert N: **Function-related replacement of bacterial siderophore pathways.** *ISME J* 2018, **12**:320-329, <https://doi.org/10.1038/ismej.2017.137>
15. Cafaro MJ, Poulsen M, Little AEF, Price SL, Gerardo NM, Wong B, Stuart AE, Larget B, Abbot P, Currie CR: **Specificity in the symbiotic association between fungus-growing ants and protective *Pseudonocardia* bacteria.** *Proc Biol Sci* 2011, **278**:1814-1822, <https://doi.org/10.1098/rspb.2010.2118>
16. Cavalleri B, Pagani H, Volpe G, Selva E, Parenti F: **A-16686, a new antibiotic from Actinoplanes. I. Fermentation, isolation and preliminary physico-chemical characteristics.** *J Antibiot* 1984, **37**:309-317, <https://doi.org/10.7164/antibiotics.37.309>
17. Chase AB, Sweeney D, Muskat MN, Guillén-Matus DG, Jensen PR: **Vertical inheritance facilitates interspecies diversification in biosynthetic gene clusters and specialized metabolites.** *mBio* 2021, **12**:e02700-21, <https://doi.org/10.1128/mBio.02700-21>
18. Chevrette MG, Currie CR: **Emerging evolutionary paradigms in antibiotic discovery.** *J Ind Microbiol Biotechnol* 2019, **46**:257-271, <https://doi.org/10.1007/s10295-018-2085-6>
19. Cohen DR, Townsend CA: **A dual role for a polyketide synthase in dynemicin enediyne and anthraquinone biosynthesis.** *Nat Chem* 2018, **10**:231-236, <https://doi.org/10.1038/nchem.2876>
20. Combes P, Till R, Bee S, Smith MCM: **The *Streptomyces* genome contains multiple pseudo-attB sites for the ϕ C31-encoded site-specific recombination system.** *J Bacteriol* 2002, **184**:5746-5752, <https://doi.org/10.1128/JB.184.20.5746-5752.2002>
21. Coronelli C, White RJ, Lancini GC, Parenti F: **Lipiarmycin, a new antibiotic from Actinoplanes. II. Isolation, chemical, biological and biochemical characterization.** *J Antibiot* 1975, **28**:253-259, <https://doi.org/10.7164/antibiotics.28.253>
22. Cross KL, Campbell JH, Balachandran M, Campbell AG, Cooper CJ, Griffen A, Heaton M, Joshi S, Klingeman D, Leys E, Yang Z, Parks JM, Podar M: **Targeted isolation and cultivation of uncultivated bacteria by reverse genomics.** *Nat Biotechnol* 2019, **37**:1314-1321, <https://doi.org/10.1038/s41587-019-0260-6>
23. de la Cruz M, González I, Parish CA, Onishi R, Tormo JR, Martín J, Peláez F, Zink D, El Aouad N, Reyes F, Genilloud O, Vicente F: **Production of ramoplanin and ramoplanin analogs by actinomycetes.** *Front Microbiol* 2017, **8**:343.
24. Deng Y, Zhang X, Zhang X: **Recent advances in genetic modification systems for Actinobacteria.** *Appl Microbiol Biotechnol* 2017, **101**:2217-2226, <https://doi.org/10.1007/s00253-017-8156-1>
25. Ding T, Yang L-J, Zhang W-D, Shen Y-H: **The secondary metabolites of rare actinomycetes: chemistry and bioactivity.** *RSC Adv* 2019, **9**:21964-21988, <https://doi.org/10.1039/C9RA03579F>
26. Dinós GP: **The macrolide antibiotic renaissance.** *Br J Pharmacol* 2017, **174**:2967-2983, <https://doi.org/10.1111/bph.13936>
27. Doroghazi JR, Albright JC, Goering AW, Ju K-S, Haines RR, Tchaluikov KA, Labeda DP, Kelleher NL, Metcalf WW: **A roadmap for natural product discovery based on large-scale genomics and metabolomics.** *Nat Chem Biol* 2014, **10**:963-968, <https://doi.org/10.1038/nchembio.1659>
28. Duncan KR, Crüsemann M, Lechner A, Sarkar A, Li J, Ziemert N, Wang M, Bandeira N, Moore BS, Dorrestein PC, Jensen PR: **Molecular networking and pattern-based genome mining improves discovery of biosynthetic gene clusters and their products from *Salinispora* species.** *Chem Biol* 2015, **22**:460-471, <https://doi.org/10.1016/j.chembiol.2015.03.010>
29. Erb W, Zhu J: **From natural product to marketed drug: the tiacumicin odyssey.** *Nat Prod Rep* 2012, **30**:161-174, <https://doi.org/10.1039/C2NP20080E>
30. Ezeobiora CE, Igbokwe NH, Amin DH, Enwuru NV, Okpalanwa CF, Mendie UE: **Uncovering the biodiversity and biosynthetic potentials of rare actinomycetes.** *Future J Pharm Sci* 2022, **8**:23, <https://doi.org/10.1186/s43094-022-00410-y>
31. Feeney MA, Newitt JT, Addington E, Algora-Gallardo L, Allan C, Balis L, Birke AS, Castañón-Espriu L, Charkoudian LK, Devine R, Gayrard D, Hamilton J, Hennrich O, Hoskisson PA, Keith-Baker M, Klein JG, Kruasawan W, Mark DR, Mast Y, McHugh RE, McLean TC, Mohit E, Munnoch JT, Murray J, Noble K, Otani H, Parra J, Pereira CF, Perry L, Pintor-Escobar L, Pritchard L, Prudence SMM, Russell AH, Schniete JK, Seipke RF, Sélem-Mojica N, Undabarrena A, Vind K, van Wezel GP, Wilkinson B, Worsley SF, Duncan KR, Fernández-Martínez LT, Hutchings MI: **ActinoBase: tools and protocols for researchers working on *Streptomyces* and other filamentous actinobacteria.** *Microb Genom* 2022, **8**:mgen000824, <https://doi.org/10.1099/mgen.0.000824>.
- Using a community approach, a platform to share practical resources and knowledge has been created, the authors showcase the potential of this to accelerate research on understudied strains.
32. Feling RH, Buchanan GO, Mincer TJ, Kauffman CA, Jensen PR, Fenical W: **Salinosporamide A: a highly cytotoxic proteasome inhibitor from a novel microbial source, a marine bacterium of the new genus *Salinispora*.** *Angew Chem Int Ed Engl* 2003, **42**:355-357, <https://doi.org/10.1002/anie.200390115>
33. Floss HG, Yu T-W: **Rifamycin Mode of action, resistance, and biosynthesis.** *Chem Rev* 2005, **105**:621-632, <https://doi.org/10.1021/cr030112j>
34. Gavriilidou A, Kautsar SA, Zaburanyi N, Krug D, Müller R, Medema MH, Ziemert N: **Compendium of specialized metabolite biosynthetic diversity encoded in bacterial genomes.** *Nat Microbiol* 2022, **7**:726-735, <https://doi.org/10.1038/s41564-022-01110-2>.
- The authors estimated only three percent of natural products gene clusters have been experimentally characterised. To do this they analysed ~170,000 bacterial genomes and ~47,000 metagenome assembled genomes (MAGs). They also used clustering to show that biosynthetic diversity drops significantly at the genus level, identifying it as an appropriate taxonomic rank for comparison.
35. González-Salazar LA, Quezada M, Rodríguez-Orduña L, Ramos-Aboites H, Capon RJ, Souza-Saldivar V, Barona-Gomez F, Licona-Cassani C: **Biosynthetic novelty index reveals the metabolic potential of rare actinobacteria isolated from highly oligotrophic sediments.** *Microb Genom* 2023, **9**:000921, <https://doi.org/10.1099/mgen.0.000921>.
- By using a genomic metric called the BiNI, the authors compared and ranked (according to novelty) genomes from strains of different taxonomic and ecological backgrounds, finding that rare actinobacteria encoded more BGC novelty.
36. Goodfellow M, Williams ST: **Ecology of actinomycetes.** *Ann Rev Microbiol* 1983, **37**:189-216.
37. Gregory MA, Till R, Smith MCM: **Integration site for *Streptomyces* phage phiBT1 and development of site-specific integrating vectors.** *J Bacteriol* 2003, **185**:5320-5323, <https://doi.org/10.1128/JB.185.17.5320-5323.2003>
38. Hamm JN, Erdmann S, Eloë-Fadrosch EA, Angeloni A, Zhong L, Brownlee C, Williams TJ, Barton K, Carswell S, Smith MA, Brazendale S, Hancock AM, Allen MA, Raftery MJ, Cavicchioli R: **Unexpected host dependency of Antarctic *Nanohaloarchaeota*.** *Proc Natl Acad Sci* 2019, **116**:14661-14670, <https://doi.org/10.1073/pnas.1905179116>
39. Hayakawa M: **Studies on the isolation and distribution of rare actinomycetes in soil.** *Actinomycetologica* 2008, **22**:12-19, <https://doi.org/10.3209/saj.SAJ220103>
40. Heng E, Tan LL, Zhang MM, Wong FT: **CRISPR-Cas strategies for natural product discovery and engineering in actinomycetes.** *Process Biochem* 2021, **102**:261-268, <https://doi.org/10.1016/j.procbio.2021.01.007>

41. Hifnawy MS, Fouda MM, Sayed AM, Mohammed R, Hassan HM, AbouZid SF, Rateb ME, Keller A, Adamek M, Ziemert N, Abdelmohsen UR: **The genus *Micromonospora* as a model microorganism for bioactive natural product discovery**. *RSC Adv* 2020, **10**:20939-20959, <https://doi.org/10.1039/D0RA04025H>
42. Hutchings MI, Truman AW, Wilkinson B: **Antibiotics: past, present and future**. *Curr Opin Microbiol Antimicrob* 2019, **51**:72-80, <https://doi.org/10.1016/j.mib.2019.10.008>
43. Jensen PR: **Microbe profile: *Salinispora tropica*: natural products and the evolution of a unique marine bacterium**. *Microbiol Read Engl* 2022, **168**:001163, <https://doi.org/10.1099/mic.0.001163>
44. Jensen PR, Moore BS, Fenical W: **The marine actinomycete genus *Salinispora*: a model organism for secondary metabolite discovery**. *Nat Prod Rep* 2015, **32**:738-751, <https://doi.org/10.1039/c4np00167b>
45. Kalkreuter E, Pan G, Cepeda AJ, Shen B: **Targeting bacterial genomes for natural product discovery**. *Trends Pharmacol Sci* 2020, **41**:13-26, <https://doi.org/10.1016/j.tips.2019.11.002>
46. Kautsar SA, van der Hoof JJJ, de Ridder D, Medema MH: **BiG-SLiCE: a highly scalable tool maps the diversity of 1.2 million biosynthetic gene clusters**. *GigaScience* 2021, **10**:giaa154, <https://doi.org/10.1093/gigascience/giaa154>
- The authors clustered 1,225,071 BGCs collected from over 200,000 publicly available microbial genomes and metagenome-assembled genomes to analyse diversity and how this compared across taxa. In this work they also assessed cultivated and un-cultivated taxa to quantify underexplored biosynthetic potential showing that > 96% of the BGCs in their dataset were uncharacterized. They also demonstrated that rare actinomycete taxa, such as *Saccharopolyspora*, *Nocardia* and *Amycolatopsis* have considerable biosynthetic potential.
47. Kieser T, Bibb M, Chater K, Butter M, Hopwood D, Bittner M, Buttner M: **Practical *Streptomyces* Genetics: A Laboratory Manual**. John Innes Foundation; 2000.
48. Kobayashi M, Sone M, Umemura M, Nabeshima T, Nakashima T, Hellström S: **Comparisons of cochleotoxicity among three gentamicin compounds following intratympanic application**. *Acta Otolaryngol* 2008, **128**:245-249, <https://doi.org/10.1080/00016480701558948>
49. Kost C, Lakatos T, Böttcher I, Arendholz W-R, Redenbach M, Wirth R: **Non-specific association between filamentous bacteria and fungus-growing ants**. *Naturwissenschaften* 2007, **94**:821-828, <https://doi.org/10.1007/s00114-007-0262-y>
50. Lazzarini A, Cavaletti L, Toppo G, Marinelli F: **Rare genera of actinomycetes as potential producers of new antibiotics**. *Antonie Van Leeuwenhoek* 2000, **78**:399-405, <https://doi.org/10.1023/A:1010287600557>
51. Letzel A-C, Li J, Amos GCA, Millán-Aguiñaga N, Ginigini J, Abdelmohsen UR, Gaudêncio SP, Ziemert N, Moore BS, Jensen PR: **Genomic insights into specialized metabolism in the marine actinomycete *Salinispora***. *Environ Microbiol* 2017, **19**:3660-3673, <https://doi.org/10.1111/1462-2920.13867>
52. Li J, Wang B, Yang Q, Si H, Zhao Y, Zheng Y, Peng W: **Enabling efficient genetic manipulations in a rare actinomycete *Pseudonocardia alni* Shahu**. *Front Microbiol* 2022, **13**:848964.
53. Lin X, Lu J, Yang M, Dong BR, Wu HM: **Macrolides for diffuse panbronchiolitis**. *Cochrane Database Syst Rev* 2015, **2015**:CD007716, <https://doi.org/10.1002/14651858.CD007716.pub4>
54. Liu C, Zhang Z, Fukaya K, Urabe D, Harunari E, Oku N, Igarashi Y: **Catellatolactams A-C, plant growth-promoting ansamacrolactams from a rare actinomycete of the genus *Catellatospora***. *J Nat Prod* 2022, **85**:1993-1999.
- Three novel ansamacrolactams were isolated from the rare actinomycete genus *Catellatospora*. In addition to showcasing the chemical potential of a very understudied genus, they found that the metabolites isolated had utility in plant growth promotion.
55. Marcone GL, Foulston L, Binda E, Marinelli F, Bibb M, Beltrametti F: **Methods for the genetic manipulation of *Nonomuraea* sp. ATCC 39727**. *J Ind Microbiol Biotechnol* 2010, **37**:1097-1103, <https://doi.org/10.1007/s12029-010-0807-5>
56. McCafferty DG, Cudic P, Frankel BA, Barkallah S, Kruger RG, Li W: **Chemistry and biology of the ramoplanin family of peptide antibiotics**. *Pept Sci* 2002, **66**:261-284, <https://doi.org/10.1002/bip.10296>
57. McClung DJ, Du Y, Antonich DJ, Bonet B, Zhang W, Traxler MF: **Harnessing rare actinomycete interactions and intrinsic antimicrobial resistance enables discovery of an unusual metabolic inhibitor**. *mBio* 2022, **13**:e00393-22, <https://doi.org/10.1128/mbio.00393-22>
- Antimicrobial interactions yielded the discovery of a family of antimicrobials named the dynaplanins produced by the rare actinomycete *Couchioplanes caeruleus*. This work highlights to main challenges, the first is that the heterologous expression for downstream elucidation of the BGC was carried out using *Streptomyces*, the second was that by using a genome mining approach, the BGC would have been undetected.
58. McCormick MH, McGuire JM, Pittenger GE, Pittenger RC, Stark WM: **Vancomycin, a new antibiotic. I. Chemical and biologic properties**. *Antibiot Annu* 1955, **3**:606-611.
59. McGuire JM, Bunch RL, Anderson RC, Boaz HE, Flynn EH, Powell HM, Smith JW: **Ilotycin, a new antibiotic**. *Antibiot Chemother North Ill* 1952, **2**:281-283.
60. Mitousis L, Thoma Y, Musiol-Kroll EM: **An update on molecular tools for genetic engineering of actinomycetes—the source of important antibiotics and other valuable compounds**. *Antibiotics* 2020, **9**:494, <https://doi.org/10.3390/antibiotics9080494>
61. Mosaei H, Molodtsov V, Kepplinger B, Harbottle J, Moon CW, Jeeves RE, Ceccaroni L, Shin Y, Morton-Laing S, Marrs ECL, Wills C, Clegg W, Yuzenkova Y, Perry JD, Bacon J, Errington J, Allenby NEE, Hall MJ, Murakami KS, Zenkin N: **Mode of action of kanglemycin A, an ansamycin natural product that is active against rifampicin-resistant *Mycobacterium tuberculosis***. *Mol Cell* 2018, **72**:263-274.e5, <https://doi.org/10.1016/j.molcel.2018.08.028>
62. Mullane K, Lee C, Bressler A, Buitrago M, Weiss K, Dabovic K, Praestgaard J, Leeds JA, Blais J, Pertel P: **Multicenter, randomized clinical trial to compare the safety and efficacy of LFF571 and vancomycin for *Clostridium difficile* infections**. *Antimicrob Agents Chemother* 2015, **59**:1435-1440, <https://doi.org/10.1128/AAC.04251-14>
63. Musiol-Kroll EM, Tocchetti A, Sosio M, Stegmann E: **Challenges and advances in genetic manipulation of filamentous actinomycetes – the remarkable producers of specialized metabolites**. *Nat Prod Rep* 2019, **36**:1351-1369, <https://doi.org/10.1039/C9NP00029A>
64. Nah H-J, Pyeon H-R, Kang S-H, Choi S-S, Kim E-S: **Cloning and heterologous expression of a large-sized natural product biosynthetic gene cluster in *Streptomyces* species**. *Front Microbiol* 2017, **8**:394.
65. Nazari B, Forneris CC, Gibson MI, Moon K, Schramma KR, Seyedsayamdost MR: ***Nonomuraea* sp. ATCC 55076 harbours the largest actinomycete chromosome to date and the kistamicin biosynthetic gene cluster**. *MedChemComm* 2017, **8**:780-788, <https://doi.org/10.1039/C6MD00637J>
66. Oren A, Garrity GM: **Valid publication of the names of forty-two phyla of prokaryotes**. *Int J Syst Evol Microbiol* 2021, **71**:005056, <https://doi.org/10.1099/ijsem.0.005056>
67. Parenti F, Beretta G, Berti M, Arioli V: **Teichomycins, new antibiotics from *Actinoplanes teichomyceticus* Nov. Sp. I. Description of the producer strain, fermentation studies and biological properties**. *J Antibiot* 1978, **31**:276-283, <https://doi.org/10.7164/antibiotics.31.276>
68. Parenti F, Pagani H, Beretta G: **Lipiarmycin, a new antibiotic from *Actinoplanes*. I. Description of the producer strain and fermentation studies**. *J Antibiot* 1975, **28**:247-252, <https://doi.org/10.7164/antibiotics.28.247>
69. Peek J, Lilic M, Montiel D, Milshteyn A, Woodworth I, Biggins JB, Ternei MA, Calle PY, Danziger M, Warrior T, Saito K, Braffman N,

- Fay A, Glickman MS, Darst SA, Campbell EA, Brady SF: **Rifamycin congeners kanglemycins are active against rifampicin-resistant bacteria via a distinct mechanism.** *Nat Commun* 2018, **9**:4147, <https://doi.org/10.1038/s41467-018-06587-2>
70. Petrosillo N, Granata G, Cataldo MA: **Novel antimicrobials for the treatment of *Clostridium difficile* infection.** *Front Med* 2018, **5**:96, <https://doi.org/10.3389/fmed.2018.00096>
71. Prudence SMM, Newitt JT, Worsley SF, Macey MC, Murrell JC, Lehtovirta-Morley LE, Hutchings MI: **Soil, senescence and exudate utilisation: characterisation of the Paragon var. spring bread wheat root microbiome.** *Environ Micro* 2021, **16**:12, <https://doi.org/10.1186/s40793-021-00381-2>
72. Pruitt KD, Tatusova T, Maglott DR: **NCBI reference sequences (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins.** *Nucleic Acids Res* 2007, **35**:D61-D65, <https://doi.org/10.1093/nar/gkl842>
73. Qin Z, Munnoch T, Devine J, A. Holmes R, F. Seipke N, A. Wilkinson R, Wilkinson K, I. Hutchings B, M: **Formicamycins, antibacterial polyketides produced by *Streptomyces formicae* isolated from African *Tetraponera* plant-ants.** *Chem Sci* 2017, **8**:3218-3227, <https://doi.org/10.1039/C6SC04265A>
74. Riahi HS, Heidarieh P, Fatahi-Bafghi M: **Genus *Pseudonocardia*: What we know about its biological properties, abilities and current application in biotechnology.** *J Appl Microbiol* 2022, **132**:890-906, <https://doi.org/10.1111/jam.15271>
75. Román-Ponce B, Millán-Aguñaga N, Guillen-Matus D, Chase AB, Ginigini JGM, Soapi K, Feussner KD, Jensen PR, Trujillo ME: **Six novel species of the obligate marine actinobacterium *Salinispora*, *Salinispora cortesiana* sp. nov., *Salinispora fenicalii* sp. nov., *Salinispora goodfellowii* sp. nov., *Salinispora mooreana* sp. nov., *Salinispora oceanensis* sp. nov. and *Salinispora vitiensis* sp. nov., and emended description of the genus *Salinispora*.** *Int J Syst Evol Microbiol* 2020, **70**:4668-4682, <https://doi.org/10.1099/ijsem.0.004330>
76. Sayed AM, Abdel-Wahab NM, Hassan HM, Abdelmohsen UR: **Saccharopolyspora: an underexplored source for bioactive natural products.** *J Appl Microbiol* 2020, **128**:314-329, <https://doi.org/10.1111/jam.14360>
77. Schorn MA, Alanjary MM, Aguinaldo K, Korobeynikov A, Podell S, Patin N, Lincecum T, Jensen PR, Ziemert N, Moore BS: **Sequencing rare marine actinomycete genomes reveals high density of unique natural product biosynthetic gene clusters.** *Microbiology* 2016, **162**:2075-2086, <https://doi.org/10.1099/mic.0.000386>
78. Seipke RF, Barke J, Brearley C, Hill L, Yu DW, Goss RJM, Hutchings MI: **A single *Streptomyces* symbiont makes multiple antifungals to support the fungus farming ant *Acromyrmex octospinosus*.** *PLoS One* 2011, **6**:e22028, <https://doi.org/10.1371/journal.pone.0022028>
79. Seipke RF, Barke J, Heavens D, Yu DW, Hutchings MI: **Analysis of the bacterial communities associated with two ant-plant symbioses.** *MicrobiologyOpen* 2013, **2**:276-283, <https://doi.org/10.1002/mbo3.73>
80. Seipke RF, Kaltenpoth M, Hutchings MI: ***Streptomyces* as symbionts: an emerging and widespread theme?** *FEMS Microbiol Rev* 2012, **36**:862-876, <https://doi.org/10.1111/j.1574-6976.2011.00313.x>
81. Sen R, Ishak HD, Estrada D, Dowd SE, Hong E, Mueller UG: **Generalized antifungal activity and 454-screening of *Pseudonocardia* and *Amycolatopsis* bacteria in nests of fungus-growing ants.** *Proc Natl Acad Sci* 2009, **106**:17805-17810, <https://doi.org/10.1073/pnas.0904827106>
82. Sensi P, Greco AM, Ballotta R: **Rifomycin. I. Isolation and properties of rifomycin B and rifomycin complex.** *Antibiot Annu* 1959, **7**:262-270.
83. Seshadri R, Roux S, Huber KJ, Wu D, Yu S, Udway D, Call L, Nayfach S, Hahnke RL, Pukall R, White JR, Varghese NJ, Webb C, Palaniappan K, Reimer LC, Sardà J, Bertsch J, Mukherjee S, Reddy TBK, Hajek PP, Huntemann M, Chen I-MA, Spunde A, Clum A, Shapiro N, Wu Z-Y, Zhao Z, Zhou Y, Evtushenko L, Thijs S, Stevens V, Eloe-Fadrosh EA, Mouncey NJ, Yoshikuni Y, Whitman WB, Klenk H-P, Woyke T, Göker M, Kyrpides NC, Ivanova NN: **Expanding the genomic encyclopedia of Actinobacteria with 824 isolate reference genomes.** *Cell Genom* 2022, **2**:100213, <https://doi.org/10.1016/j.xgen.2022.100213>
- This study evaluates 824 actinomycetota genomes from diverse environments, they show that a third of the taxonomic diversity in this genus is not represented genomically. This represents a significant dataset, where the authors show niche-specific functional adaptations, illustrating the potential of underexplored environments and taxa.
84. Shen Q, Dai G, Ravichandran V, Liu Y, Zhong L, Sui H, Ren X, Jiao N, Zhang Y, Zhou H, Bian X: **Saccharochelins A-H, cytotoxic amphiphilic siderophores from the rare marine actinomycete *Saccharothrix* sp. D09.** *J Nat Prod* 2021, **84**:2149-2156, <https://doi.org/10.1021/acs.jnatprod.1c00155>
- This study shows the chemical and biomedical potential of the rare-actinomycete genus *Saccharothrix*, the authors isolated eight amphiphilic siderophores, saccharochelins A-H and demonstrated their anticancer potential.
85. Siegl T, Petzke L, Welle E, Luzhetskyy A: **I-SceI endonuclease: a new tool for DNA repair studies and genetic manipulations in streptomycetes.** *Appl Microbiol Biotechnol* 2010, **87**:1525-1532, <https://doi.org/10.1007/s00253-010-2643-y>
86. Soldatou S, Eldjárn GH, Ramsay A, van der Hooff JJJ, Hughes AH, Rogers S, Duncan KR: **Comparative metabologenomics analysis of polar actinomycetes.** *Mar Drugs* 2021, **19**:103, <https://doi.org/10.3390/md19020103>
- Metabolite extracts of 25 Polar, rare actinomycete strains, including the genus *Pseudonocardia* showed promising bioactivity against drug-resistant bacterial pathogens such as *Klebsiella pneumoniae* and *Acinetobacter baumannii*.
87. Subramani R, Sipkema D: **Marine rare actinomycetes: a promising source of structurally diverse and unique novel natural products.** *Mar Drugs* 2019, **17**:249, <https://doi.org/10.3390/md17050249>
88. Terlou BR, Blin K, Navarro-Muñoz JC, Avalon NE, Chevrette MG, Egbert S, Lee S, Meijer D, Recchia MJJ, Reitz ZL, van Santen JA, Selem-Mojica N, Tørring T, Zaroubi L, Alanjary M, Aleti G, Aguilar C, Al-Salih SAA, Augustijn HE, Avelar-Rivas JA, Avitia-Domínguez LA, Barona-Gómez F, Bernaldo-Agüero J, Bielinski VA, Biermann F, Booth TJ, Carrion Bravo VJ, Castelo-Branco R, Chagas FO, Cruz-Morales P, Du C, Duncan KR, Gavriilidou A, Gayraud D, Gutiérrez-García K, Haslinger K, Helfrich EJJ, van der Hooff JJJ, Jati AP, Kalkreuter E, Kalyvas N, Kang KB, Kautsar S, Kim W, Kunjapur AM, Li Y-X, Lin G-M, Loureiro C, Louwen JJR, Louwen NLL, Lund G, Parra J, Philmus B, Pourmohsenin B, Pronk LJ, Rego A, Rex DAB, Robinson S, Rosas-Becerra LR, Roxborough ET, Schorn MA, Scobie DJ, Singh KS, Sokolova N, Tang X, Udway D, Vigneshwari A, Vind K, Vromans SPJM, Waschulin V, Williams SE, Winter JM, Witte TE, Xie H, Yang D, Yu J, Zdouc M, Zhong Z, Collemare J, Lington RG, Weber T, Medema MH: **MIBiG 3.0: a community-driven effort to annotate experimentally validated biosynthetic gene clusters.** *Nucleic Acids Res* 2023, **51**:D603-D610, <https://doi.org/10.1093/nar/gkac1049>
- MIBiG 3.0 has over 1,000 BGCs corresponding to known products across the phyla Actinomycetota, the authors also showcase the utility of matching bioactivity to genes and metabolites.
89. Theriault RJ, Karwowski JP, Jackson M, Girolami RL, Sunga GN, Vojtko CM, Coen LJ: **Tiacumicins, a novel complex of 18-membered macrolide antibiotics. I. Taxonomy, fermentation and antibacterial activity.** *J Antibiot* 1987, **40**:567-574, <https://doi.org/10.7164/antibiotics.40.567>
90. Tiwari K, Gupta RK: **Diversity and isolation of rare actinomycetes: an overview.** *Crit Rev Microbiol* 2013, **39**:256-294, <https://doi.org/10.3109/1040841X.2012.709819>
91. Tong Y, Weber T, Lee SY: **CRISPR/Cas-based genome engineering in natural product discovery.** *Nat Prod Rep* 2019, **36**:1262-1280, <https://doi.org/10.1039/C8NP00089A>
92. Tuttle RN, Demko AM, Patin NV, Kapono CA, Donia MS, Dorrestein P, Jensen PR: **Detection of natural products and their producers in ocean sediments.** *Appl Environ Microbiol* 2019, **85**:e02830-18, <https://doi.org/10.1128/AEM.02830-18>

93. van der Meij A, Worsley SF, Hutchings MI, van Wezel GP: **Chemical ecology of antibiotic production by actinomycetes.** *FEMS Microbiol Rev* 2017, **41**:392-416, <https://doi.org/10.1093/femsre/fux005>
94. van Santen JA, Poynton EF, Iskakova D, McMan E, Alsup TA, Clark TN, Fergusson CH, Fewer DP, Hughes AH, McCadden CA, Parra J, Soldatou S, Rudolf JD, Janssen EM-L, Duncan KR, Linington RG: **The Natural Products Atlas 2.0: a database of microbially-derived natural products.** *Nucleic Acids Res* 2022, **50**:D1317-D1323, <https://doi.org/10.1093/nar/gkab941>.
This is the gold standard database of isolated and characterised microbial natural products, comprising over 32,000 entries. In this paper they illustrate ranking chemistry by taxa, which has considerable applications for informed biodiscovery efforts.
95. Vitiello L, Tibaudo L, Pegoraro E, Bello L, Canton M: **Teaching an old molecule new tricks: drug repositioning for duchenne muscular dystrophy.** *Int J Mol Sci* 2019, **20**:6053, <https://doi.org/10.3390/ijms20236053>
96. Ward AC, Allenby NE: **Genome mining for the search and discovery of bioactive compounds: the *Streptomyces* paradigm.** *FEMS Microbiol Lett* 2018, **365**:fny240, <https://doi.org/10.1093/femsle/fny240>
97. Weber FH, Richards RD, McCallum RW: **Erythromycin: a motilin agonist and gastrointestinal prokinetic agent.** *Am J Gastroenterol* 1993, **88**:485-490.
98. Weinstein MJ, Luedemann GM, Oden EM, Wagman GH: **Gentamicin, a new broad-spectrum antibiotic complex.** *Antimicrob Agents Chemother* 1963, **161**:1-7.
99. WHO: **Model list of essential medicines.** 22nd List. 2021. URL (<https://www.who.int/publications-detail-redirect/WHO-MHP-HPS-EML-2021.02>) (accessed 3.9.23). World Health Organisation. WHO/MHP/HPS/EML/2021.02.
100. Wolf T, Gren T, Thieme E, Wibberg D, Zemke T, Pühler A, Kalinowski J: **Targeted genome editing in the rare actinomycete *Actinoplanes* sp. SE50/110 by using the CRISPR/Cas9 System.** *J Biotechnol* 2016, **231**:122-128, <https://doi.org/10.1016/j.jbiotec.2016.05.039>
101. Worsley SF, Innocent TM, Holmes NA, Al-Bassam MM, Schiott M, Wilkinson B, Murrell JC, Boomsma JJ, Yu DW, Hutchings MI: **Competition-based screening helps to secure the evolutionary stability of a defensive microbiome.** *BMC Biol* 2021, **19**:205, <https://doi.org/10.1186/s12915-021-01142-w>.
The authors employ a series of RNA sequencing, bioassays, and competition experiments to show that antibacterials produced by the rare actinomycete genus *Pseudonocardia* differentially reduce the growth rates of other microbes. This work has broader implications for understanding symbiotic relationships, especially in the context of anti-bacterial discovery.
102. Xu Z, Ji L, Tang W, Guo L, Gao C, Chen X, Liu J, Hu G, Liu L: **Metabolic engineering of *Streptomyces* to enhance the synthesis of valuable natural products.** *Eng Microbiol* 2022, **2**:100022, <https://doi.org/10.1016/j.engmic.2022.100022>
103. Yaginuma S, Morishita A, Ishizawa K, Murofushi S, Hayashi M, Mutoh N: **Sporeamicin A, a new macrolide antibiotic. I. Taxonomy, fermentation, isolation and characterization.** *J Antibiot* 1992, **45**:599-606, <https://doi.org/10.7164/antibiotics.45.599>
104. Yan S, Zeng M, Wang H, Zhang H: **Micromonospora: a prolific source of bioactive secondary metabolites with therapeutic potential.** *J Med Chem* 2022, **65**:8735-8771, <https://doi.org/10.1021/acs.jmedchem.2c00626>
105. Zhang J, Lair C, Roubert C, Amaning K, Barrio MB, Benedetti Y, Cui Z, Xing Z, Li X, Franzblau SG, Baurin N, Bordon-Pallier F, Cantaloube C, Sans S, Silve S, Blanc I, Fraisse L, Rak A, Jenner LB, Yusupova G, Yusupov M, Zhang Junjie, Kaneko T, Yang TJ, Fotouhi N, Nuernberger E, Tyagi S, Betoudji F, Upton A, Sacchetti JC, Lagrange S: **Discovery of natural-product-derived sequanamycins as potent oral anti-tuberculosis agents.** *Cell* 2023, **186**:1013-1025.e24, <https://doi.org/10.1016/j.cell.2023.01.043>.
In this work, a novel series of macrolides, the sequanamycins were shown to have in vitro and in vivo activity against Mtb. Sequanamycin A (SEQ-503) is a 14-membered ring erythromycin family macrolide produced by *Allokutzneria albata* and originally discovered in the 1960s, illustrating the potential of revisiting rare-actinomycete natural products.
106. Zhang JJ, Moore BS, Tang X: **Engineering *Salinispora tropica* for heterologous expression of natural product biosynthetic gene clusters.** *Appl Microbiol Biotechnol* 2018, **102**:8437-8446, <https://doi.org/10.1007/s00253-018-9283-z>
107. Zhang JJ, Tang X, Moore BS: **Genetic platforms for heterologous expression of microbial natural products.** *Nat Prod Rep* 2019, **36**:1313-1332, <https://doi.org/10.1039/C9NP00025A>
108. Ziemert N, Lechner A, Wietz M, Millán-Aguñaga N, Chavarria KL, Jensen PR: **Diversity and evolution of secondary metabolism in the marine actinomycete genus *Salinispora*.** *Proc Natl Acad Sci USA* 2014, **111**:E1130-1139, <https://doi.org/10.1073/pnas.1324161111>