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Current Opinion in Microbiology

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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Current Opinion in Microbiology 2023, **76**:102385

This review comes from a themed issue on **Antimicrobials**

Edited by **Toni Gabaldón** and **Luiz Carvalho**

For complete overview of the section, please refer to the article collection, "Antimicrobials 2023"

Available online 5 October 2023

https://doi.org/10.1016/j.mib.2023.102385

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 (avermectins). 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 comprehensives 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*, *Isoptericola*, *Jiangella*, *Knoellia*, *Kocuria*, *Krasilnikoviella*, *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 *Tsukamurella*.

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.

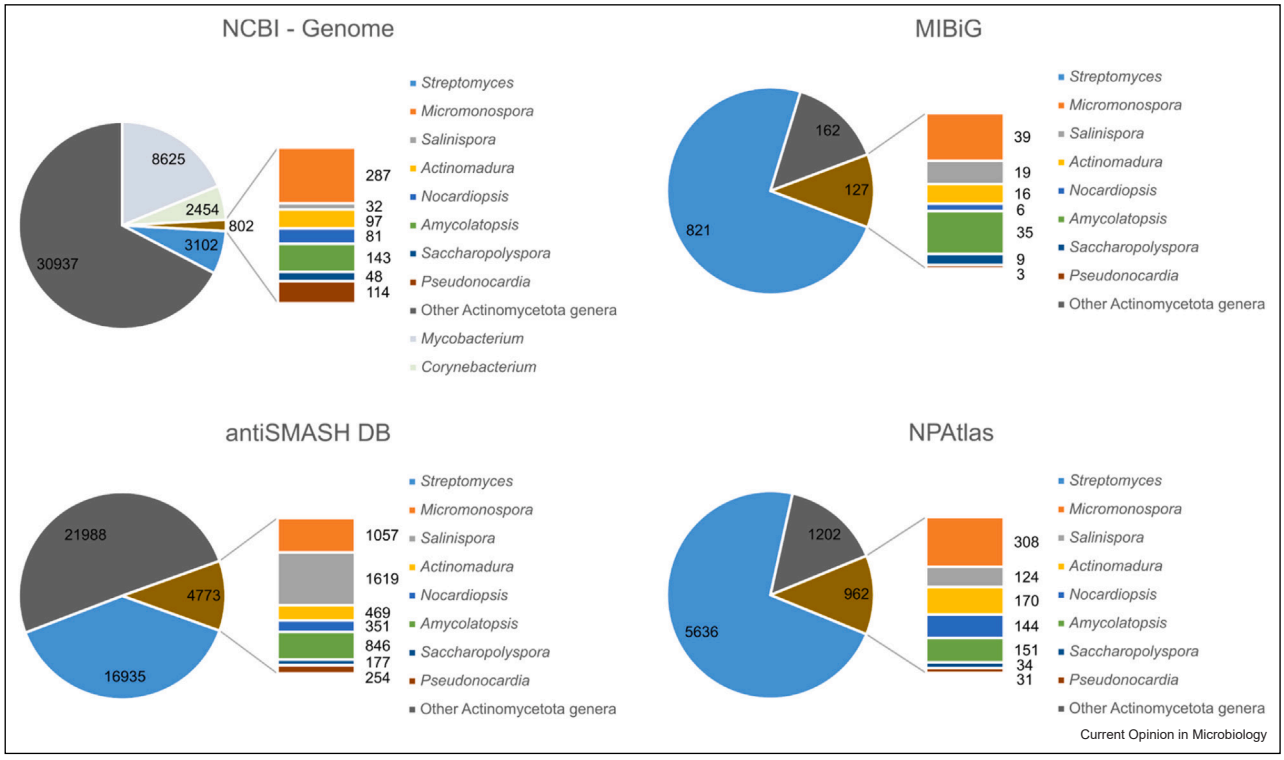
A recent survey of microbial genomes reported that *Streptomyces* encodes the greatest chemical diversity within the Actinomycetes, but that *Amycolatopsis* and *Micromonospora* also have significant biosynthetic potential [34]. As shown in Figure 1, the number of sequenced *Streptomyces* strains far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



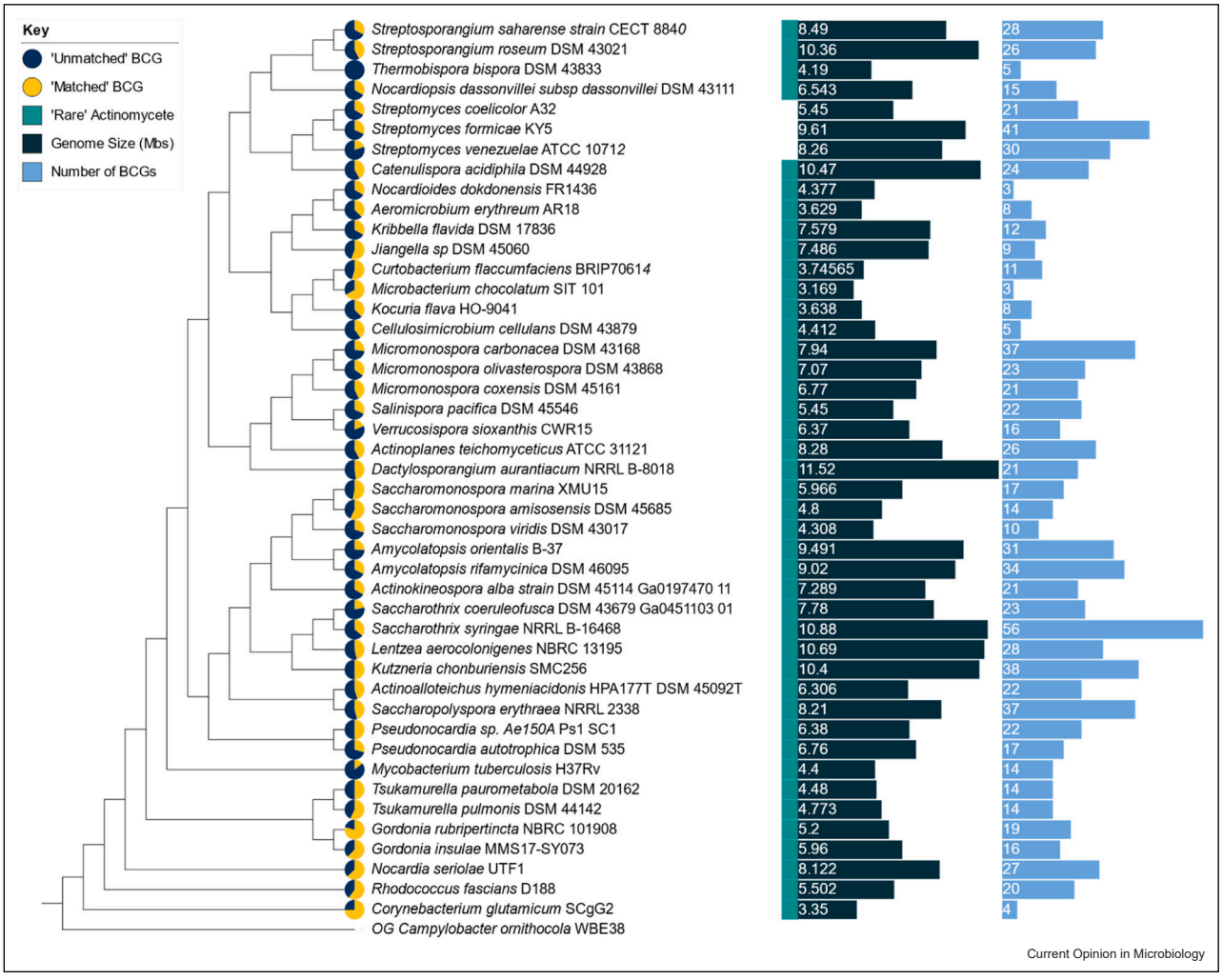
Number of entries per genus in the National Center for Biotechnology Information (NCBI) Genome, antiSMASH, MIBiG and NPAtlas databases for the phylum Actinomycetota.

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*, *Nocardiopsis*, *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

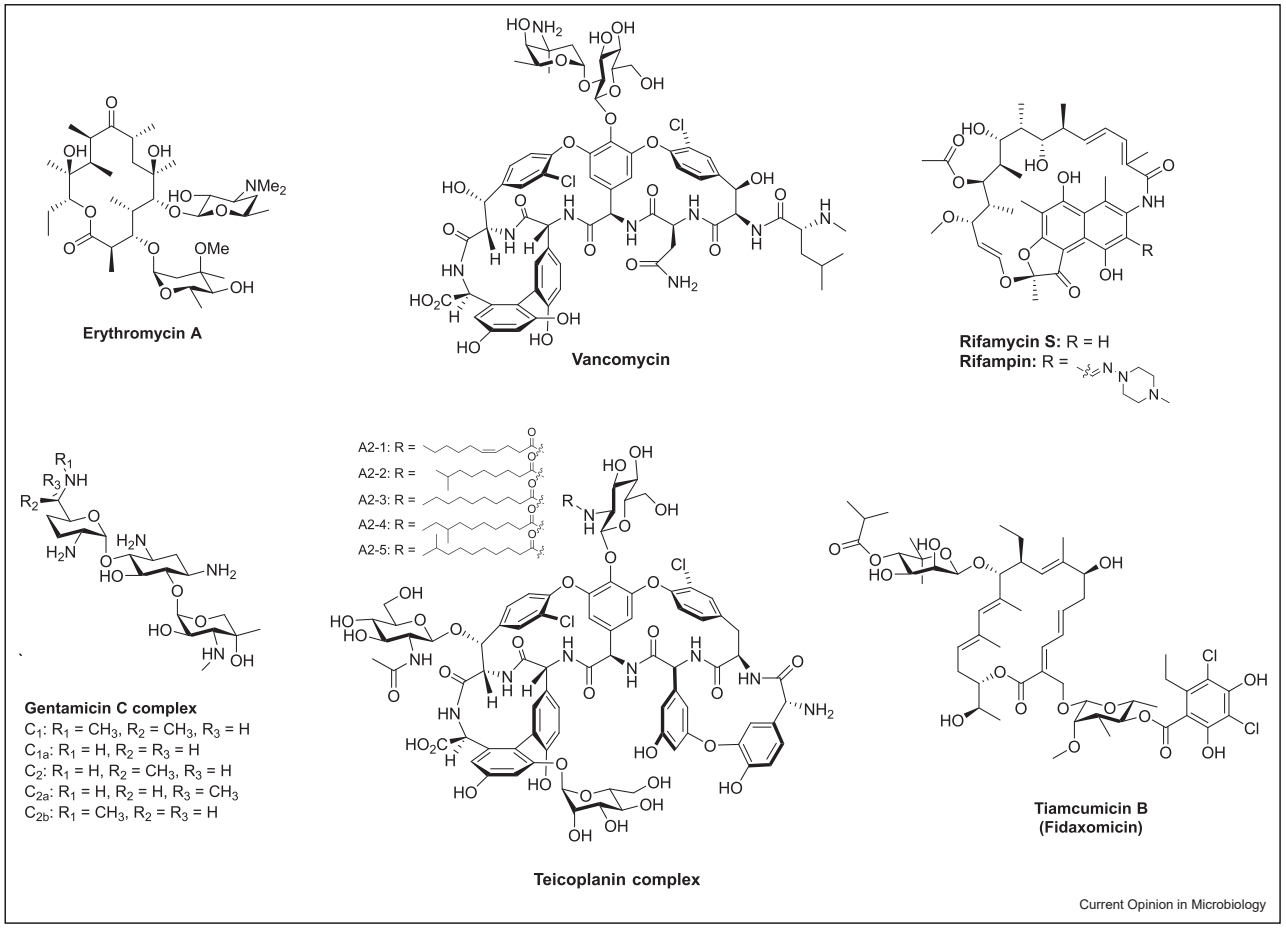


Phylogenetic tree generated by AutoMLST [1] showing genome size, number of BGCs predicted by antiSMASH and the proportion of these that are predicted to be more than 10% of a match to previously characterised clusters identified by antiSMASH (in the MIBiG database). The input to AutoMLST was 20 representitive,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

Nonomuraea sp. was recently found to harbour the largest actinomycete chromosome encoding approximately 32 BGCs [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 BGCs 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*, *Nocardiopsis*, *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 BGCs 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 BGCs 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]	*Saccharopolyspora erythraea*	Macrolide (14-atom ring)	Binds ribosome 50S subunit to inhibit protein synthesis	1952 (1952)
Vancomycin [58]	*Amycolatopsis orientalis*	Glycopeptide	Binds lipid II and inhibits cell wall synthesis	1954 (1958)
Teicoplanin [67]	*Actinoplanes teichomyceticus*	Glycopeptide	Binds lipid II and inhibits cell wall synthesis	1978 (1984)
Rifamycin [82]	*Amycolatopsis rifamycinica*	Ansamycin	Inhibits bacterial RNA polymerase to block transcription	1959 (1963)
Gentamicin [98]	*Micromonospora purpurea*	Aminoglycoside	Binds ribosome 30S subunit to inhibit protein synthesis	1963 (1964)
Fidaxomicin [68]	*Dactylosporangium aurantiacum* subsp. *hamdenesis*	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 *Actinomadura* 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 purpurera* 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. *hamdenesis*. 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.

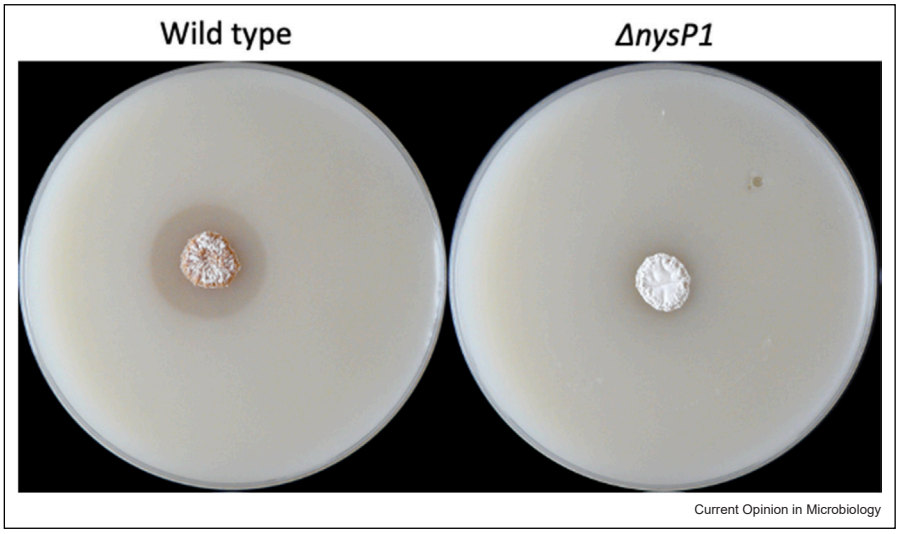
Beyond the approved drugs, peptide antibiotics produced by rare actinomycetes have proven very successful as leads for the treatment of CDI [70]. These include the glycolipodepsipeptide ramoplanin that is a cell wall-targeting antibiotic produced by *Actinoplanes ramoplaninifer* [23,56]. LFF-571 is a 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 Pallindromic 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 *nypI* 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 out 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 Nanohaloarchaeota 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.

Acknowledgements

Research into actinomycete antibiotics in the authors' laboratories was funded by grants from UK Research and Innovation (URKI) Biotechnology and Biological Sciences Research Council (BBSRC) (BB/R022054/1 to KRD), (BB/T008075/1 and BB/T014962/1 to RFS) and BB/W000628/1, BB/T015446/1, BB/T015632/1, BB/S00811X/1, BB/S009000/1 and BB/P005292/1 to MIH and BW; Medical Research Council (MRC) (G0801721 to MIH), Natural Environment Research Council (NERC) (NE/J01074X/1 to MIH, NE/M015033/1 and NE/M014657/1 to MIH and BW) and Engineering and Physical Sciences Research Council (ESPRC) (EP/X03142X/1 to KRD). KRD acknowledges funding from the UK Government Department of Environment, Food and Rural Affairs (DEFRA) through the Global Centre on Biodiversity for Climate (GCBC) (DEEPEND/DEEPEND2). MIH and BW acknowledge the Norwich Research Park and University of East Anglia for seedcorn funding and core BBSRC institute strategic program grant BB/P012523/1 to the John Innes Centre. JP received funding support from the Research Office of the University of Costa Rica (grants C2186 and C3456) for his research on actinomycetes.

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