South African actinobacteria: A treasure trove of novel bioactive metabolites for drug discovery

Although South Africa is known as one of the most biodiverse countries in the world, based on its unique plants and animals, microorganisms have received much less attention. Microorganisms in general and actinobacteria in particular are an underexplored source of new medicines. Recent studies have demonstrated the presence of diverse cultivable actinobacteria from various biomes. However, investigations of the natural product diversity associated with these microorganisms are lacking. We hereby present a review of natural products isolated from South African actinobacteria together with their biological activities. Many of these natural products are structurally novel and include compounds belonging to the following classes: anthraquinones, isoflavonoids, ketolides, macrolides, macrolactams, tripeptides and depsipeptides. They show a wide range of biological activities including antibacterial, antifungal, cytotoxic and antitumour activities.

Significance:
- This review highlights the importance of actinobacteria in the discovery of new medicines and summarizes the state-of-the-art on their research in South Africa.
- We reveal a gap in the exploitation of this resource and emphasise the opportunities for multidisciplinary research.

Introduction

Natural products from plants, invertebrates and microorganisms have played an important role in the development of new medicines and agrochemicals. Microbial natural products, in particular, offer significant advantages over natural products produced by macroorganisms. These advantages include a reduced impact on the environment (ecosystems), and hence reduced competition with food crops for arable land, as well as relative ease of production and manipulation of biosynthetic pathways to produce novel compounds for commercial exploitation.

As one of the most biodiverse countries in the world, South Africa has a rich tradition of natural products research. However, the main focus of these endeavours has been on plant natural products, and more recently marine natural products, with much less attention on microbial natural products.

One of the most important sources of medicinally important natural products are the actinobacteria. Actinobacteria, also known as actinomycetes, are filamentous Gram-positive bacteria with high guanine and cytosine (G+C) content in their DNA. The phylum Actinobacteria comprises 20 orders and more than 50 families. This bacterial phylum is widely distributed in terrestrial and both fresh and marine aquatic environments. Some can thrive under extreme conditions such as hyperaridity, high salinity, cold, high pressure, low pH (acidic) and heavy metal contaminated ecosystems. Actinobacteria are either free living, such as soil-dwelling bacteria, or living in association with other organisms, like the plant commensals or those living in and/or on the surfaces of animals like ants, termites and marine invertebrates. Some actinobacteria are also plant and animal pathogens, while others find use in agriculture, biotechnology, and medicine. In agriculture, they are saprophytic and break down dead plant and animal remains, hastening decomposition. They also aid in nitrogen fixation and are known to produce plant growth promoters, insecticides, herbicides and fungicides. Natural products produced by actinobacteria are structurally diverse and have shown diverse biological activities, including antioxidant, antimarial, antihermimic, antifungal, enzyme inhibitory, antibacterial, anticancer, immunosuppressive and cardiovascular properties. Antibiotics are the largest class of drugs discovered from actinomycetes, as they produce about 70% of all naturally derived antibiotics currently in clinical use. Most of these antibiotics were discovered during the ‘golden era’ of antibiotic drug discovery and include the aminoglycosides, β-lactams, glycopeptides, macrolides, rifamycins and tetracyclines.

In this review, we discuss the natural products produced by actinobacterial strains isolated from South African environments. The many excellent studies focusing only on the distribution and description of new actinobacteria species, as well as those only reporting on the biological activity of crude extracts and the discovery of enzymes fall outside the scope of this review.

Natural products from South African actinobacteria

Several novel actinomycete strains have been isolated from South African soils, flora and fauna, in both terrestrial and marine environments, and have been shown to contain bio-active secondary metabolites (Table 1). These strains include species of the ubiquitous Streptomyces genus and the less isolated rare genera Actinomadura, Actinosynnema, Amycolatopsis, Gordonia, Kribella, Nocardia, Nonomuraea, Rhodococcus, Streptosporangium, Saccharopolyspora and Tsukamurella.

Streptomyces

The first report of a South African actinomycete-derived secondary metabolite was a tetraene macrolide natamycin 1 (Figure 1) (also known as pimaricin, natacycin, tennecetin and E235) which was patented in 1955 for its antifungal activity. This antibiotic was first purified in 1955 from the extract of the culture broth of Streptomyces natalensis,
Table 1: Secondary metabolites from South African actinomycetes and their biological activities. Structures of compounds 1–122 are shown in the text.

<table>
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<tr>
<th>Compounds</th>
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<th>Bioactivity</th>
<th>Producing species</th>
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<td>base 62,63</td>
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Figure 1: Natamycin 1 from Streptomyces natalensis.

isolated from a soil sample collected from Pietermaritzburg (giving rise to its original name, pimaricin) in the KwaZulu-Natal Province, South Africa.24-28 Natamycin 1 was later also isolated from Streptomyces gilvosporeus, Streptomyces chattanogenesis and Streptomyces lydicus.26-27 It is active against a variety of saprophytic and parasitic fungi and is therefore used commercially as a preservative.26-28 It acts by binding to ergosterol in the fungal cell wall, thereby inhibiting fungal growth.26-28 Natamycin 1 therefore has a wide spectrum of antifungal activity and minimal toxicity to mammalian cells. It also displays in vitro activity against numerous protozoa including Trypanosoma and Acanthamoeba.31,32 Clinically, natamycin 1 is used to treat keratitis, and especially that caused by Aspergillus fumigatus, Candida albicans and Acanthamoeba sp.31,32 It is also used to treat fungal infections caused by Cephalosporium, Fusarium and Penicillium, and has shown activity against Alternaria, Colletotrichum, Curvularia, Lasiodiplodia, Scedosporium and Trichophyton.31 It is a commercial food additive which has been used for about half a century to prevent fungal growth on foods such as cheese, sausages, yoghurt, fruits, meats, baked confectioneries and beverages.31

A soil microbe antibiotic screening programme led to the isolation of actinomycete strain AB 1246E-26 from South African bushveld soil.28 Although the genus of this actinomycete strain was not determined, preliminary characterisation narrowed the taxonomic assignment to either Nocardia or the defunct Micropolyspora.33 Strain AB 1246E-26 showed activity against the antibiotic-sensitive strain of Pseudomonas aeruginosa K799/61 among other P. aeruginosa strains.33,34 The organic extract of the whole fermentation broth of strain AB 1246E-26 was
subjected to a bioactivity guided isolation protocol to yield the novel anthraquinone-derived class of antibiotics called altromycins. The altromycins A-I (Figure 2) are nine closely related members of the pluramycin class of compounds with a single epoxide substituent, an amino-disaccharide and/or a 6-deoxy-3-O-methylaltrose attached to the conjugated ring systems of an anthraquinone-γ-pyrone core.

Altromycin B was screened against 30 bacterial strains including Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus luteus, Enterococcus hirae, Streptococcus bovis, Streptococcus agalactiae, Streptococcus pyogenes, Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae, Providencia stuartii, Pseudomonas aeruginosa, Pseudomonas cepacia and Acinetobacter sp. clinical isolates. Altromycin B exhibited potent antibacterial activity against the clinical isolates of Staphylococcus and Streptococcus with a minimum inhibitory concentration (MIC) range of 0.39–3.12 µg/mL and 0.2–3.12 µg/mL, respectively, but displayed moderate to weak antibacterial activity against Gram-negative bacteria with an MIC range of 25 to >100 µg/mL. The altromycins also showed cytotoxic activity against various cancer and tumour cell lines including cervical cancer (HeLa), human lung cancer (A549), colon tumour (HCT-8), murine leukemia cell (P388) and ovarian sarcoma (M5076).

In their search for antibiotics that inhibit fatty acid biosynthesis in bacteria, researchers at Merck discovered the novel broad-spectrum antibiotic platensimycin (Figure 3) from Streptomyces platensis strain MA7327, which was originally isolated from a soil sample collected in the Eastern Cape Province of South Africa. An organic extract of the fermentation broth of S. platensis strain MA7327 was subjected to a unique antisense differential sensitivity whole-cell two-plate agar diffusion bioassay-guided fractionation process to yield platensimycin. Platensimycin consists of a 3-amino-2,4-dihydroxybenzoic acid tethered via an amide bond to a C-17 tetracyclic enone which includes a bridge-head oxygen. Another closely related compound, platencin (Figure 3), was produced by Streptomyces platensis strain MA7339 using the same bioassay-guided fractionation procedure, although its biosynthetic gene cluster was also identified in strain MA7327. Several other analogues (Figure 3) – with modifications on or loss of the aromatic ring, modifications on the terpenoid and anilide moieties and a change in the length of the enone acid portion of platensimycin and platencin – have also been isolated from strain MA7327. Compounds and 51 (Figure 3), which are structurally different from the platensimycin group of compounds, were also isolated from strain MA7327. Furthermore, other glycosylated analogues of platensimycin and platencin have...
Platensimycin has shown potent in vitro activity against both cell-free and whole-cell systems including methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus* and *Mycobacterium tuberculosis*.

Figure 3: Secondary metabolites 11–51 from South African actinomycetes *Streptomyces platensis*.
Although the isolated analogues and synthesised ones did not exhibit improved activity compared with compounds 11 and 12, they provide important structure–activity relationship information to determine the pharmacophore of 11 and 12.

In their quest to isolate biologically active metabolites from termite-associated actinobacteria, Kim et al. discovered that Streptomyces strain M56, isolated from the fungal comb of a South African Macrotermes natalensis Mn802 colony, exhibited potent broad-spectrum antifungal activity.49 Bioassay-guided isolation resulted in the purification of the novel fused bicyclic ansa macrolide natalamycin A (Figure 4) alongside other ansa macrolides including reblastatin 53, geldanamycin 54 and its derivatives, 17-O-demethyl-geldanamycin 55, 19-S-methylgeldanamycin 56, 17-amino-17-demethoxy-geldanamycin 57, methyl geldanamycinate derivative 58, 17-amino-17-demethoxy-methyl geldanamycinate 59 and 19-[(1S,5,4 R)-4′-hydroxy-1′-methoxy-2′-oxopentyl]geldanamycin 60.49 Although the fractions that yielded the isolated compounds showed strong antifungal activity against some strains, the natalamycins exhibited weak or no activity against Saccharomyces cerevisiae and other fungal isolates.49 An untargeted dereplication of the liquid chromatography–mass spectrometry (LCMS) data of the methanol extract of Streptomyces strain M56 signified the presence of new metabolites.50 The methanol extract of a large-scale culture of strain M56 was subjected to several chromatographic methods to yield the fused 5-8-5 tricyclic diterpene 17-hydroxycyclooctatin 61 (Figure 4).49 Compound 61 is a potential ERα antagonist and exhibited weak cytotoxicity activity against MCF-7 human breast cancer cell lines with an IC₅₀ value of 566.95 ± 0.48 μM.49

Further studies on the metabolites of the actinobacteria associated with the fungus-growing South African termite Macrotermes natalensis led to the isolation of Streptomyces strain RB1 which exhibited antibacterial activity against Staphylococcus aureus and Candida albicans.51 Fractionation of the methanol (MeOH) extract of strain RB1 yielded the new isoflavonoid glycosides, termisoflavone A–C 62–64, and other isoflavonoids 66–70, 72–74 (Figure 5).51 The isolated compounds showed no antifungal or antibacterial activity when screened against C. albicans, C. neoformans, S. aureus, and E. coli, but compounds 69 and 73 ameliorated cisplatin-induced kidney cell damage.51 Further investigation of the MeOH extract of strain RB1 using LCMS- and NMR-based dereplication strategies led to the identification and subsequent isolation of another new isoflavonoid glycoside, termisoflavone D 65, together with the known isoflavonoids 66, 67, 69, 71–73 and 75 (Figure 5).52 Isoflavonoid 69 displayed activity against glutamate-induced HT22 cells by preventing accumulation of intracellular reactive oxygen species.52 Another study exploring the termite associated actinobacteria for reno- and kidney-protective drug discovery found that the MeOH extract of Streptomyces sp. RB1 exhibited a protective effect against cisplatin-induced cytotoxicity.53 A bioassay (LLC-PK1 cells)-guided isolation process yielded the renoprotective 1-O-(2-aminobenzoyl)-α-L-rhamnopyranoside (ABR) 76 (Figure 5).53 Analysing the chemical and metabolomic profiles of actinobacteria derived from termite nests with an unbiased high-throughput high-performance liquid chromatography–high-resolution mass spectrometry based dereplication strategy revealed that Streptomyces sp. M41 isolated from the South African termite Macrotermes natalensis produces new complex nonribosomal peptide polyketide synthase (NRPS/PKS) hybrid compounds.54 Chromatographic purification of a large-scale culture of strain M41 interestingly yielded new analogues (two linear 77, 78 and one cyclic 79) of the cyclic depsidepeptide dentigerumycin 80 (Figure 6).54

The South African soil actinomycete, Streptomyces canus strain CA-091830, is a producer of the cyclic depsipeptides krisynomycins A–C 81–83 (Figure 7).54,55 Krisynomycin A was initially isolated based on a screening project with the aim of identifying and isolating imipenem potentiators against methicillin-resistant Staphylococcus aureus (MRSA).53 Further investigation led to the isolation of Krisynomycin B and C, which are chlorinated analogues of Krisynomycin A.54 Although compounds 81–83 showed weak activity against MRSA, the activity was improved when they were tested in combination with sub-lethal concentrations of imipenem.55

Figure 4: Secondary metabolites 52–61 from South African actinomycetes Streptomyces strain M56.
Rare actinomycete strains

The rare actinomycete *Actinomadura* sp. 5-2, which was recovered from the gut of the fungus-growing termite *M. natalensis*, produced novel, highly substituted tropolone alkaloids, rubterolones A–F 84–89 (Figure 8). These compounds were detected by both bioactivity and high-resolution mass spectrometry based dereplication techniques, and subsequently isolated from the organic extract of a culture of strain 5-2. Curiously, compounds 84–89 did not show any significant antifungal activity.

The crude extract of another *Actinomadura* isolate, strain RB99, isolated from the surface of the termite *M. natalensis*, was analysed by liquid chromatography (LC)/ultraviolet (UV)/mass spectrometry (MS) and shown to produce new compounds. A spectrometry guided isolation led to the discovery of three new cyclic tripeptides named natalenamides A–C 90–92 (Figure 9). The isolated compounds exhibited weak cytotoxicity when screened against HepG2 and HeLa/A549 cells. Compound 92 showed significant activity against IBMX-mediated melanin synthesis in a dose-dependent manner.

Analyses of the high-resolution tandem mass spectrometry (HR-MS²) data of the MeOH extract of strain RB99 and further exploration of the HR-MS² data on the Global Natural Product Social (GNPS) molecular networking platform showed that strain RB99 produces polyhalogenated isoflavonoids. Seoung et al. proved that *Actinomadura* sp. RB99 can bio-transform the plant-based daidzein 74 and genistein 75 isoflavonoids contained in the ISP2 growth medium to polyhalogenated derivatives. Optimisation of the growth...
medium (ISP2 augmented with NaCl or KBr) led to the production and subsequent MS-guided purification of eight polychlorinated analogues 93–100 (Figure 9), of which six were new, and seven novel polybrominated analogues 101–107 (Figure 9), of daidzein and genistein. The isolated chlorinated analogues did not exhibit any antibacterial or antifungal activities against E. coli, S. aureus, S. epidermidis and C. albicans, but the brominated analogues 101 and 105 were active against Helicobacter pylori. Additional analysis of the LCMS data of the MeOH extract of strain RB99 led to the detection and isolation of the antibiotic and antitumour agent fridamycin A 108 (Figure 9), which is a type II polyketide. Fridamycin A 108 showed good antidiabetic properties in 3T3-L1 adipocytes and could serve as a promising lead for type 2 diabetes drug discovery.

Metabolomic and bioactivity profiling of termite-associated actinomycetes led to the detection and subsequent isolation of four new 20-membered glycosylated polyketide macro lactams, named macrotermycins A–D 109–112 (Figure 10), from the organic extract of the rare actinomycete Amycolatopsis sp. M39. Strain M39 was also isolated from the termite M. natalensis and its organic crude extract exhibited a unique metabolomic profile and was active against the termite fungal garden competitor Pseudoxylaria spp. Only compounds 109 and 111 were active against Pseudoxylaria sp.

A rare actinomycete, Kribbella speibonae strain SK5, isolated from a soil sample collected from Stellenbosch in the Western Cape Province of South Africa, displayed strong antimycobacterial activity against Mycobacterium aurum strain A+. Chemical and metabolomic profiling of an organic extract of a liquid culture of this strain showed that it is a prolific producer of hydroxamate siderophores, including new dehydroxylated desferrioxamine analogues and diketopiperazines (DKP). Two new dehydroxylated desferrioxamines, speibonoxamine 113 and desoxy-desferrioxamine D1 114 (Figure 11), alongside already reported desferrioxamines 115–118 and a DKP 119 (Figure 11), were subsequently isolated from an organic extract of a liquid culture of strain SK5. The plausible structures of three new dehydroxylated analogues 120–122 were determined by the GNPS molecular network and MS/MS fragmentation analyses.
Conclusions and future prospects

Biodiversity is “more than just legs and leaves” and South Africa’s microbial biodiversity presents a tremendous opportunity for the natural products chemist and those interested in drug discovery. In this review, we have described 122 compounds and shown that South African actinobacteria are prolific producers of novel, bioactive metabolites. Interestingly, the first compound described from a South African actinomycete, natamycin, is also the only one that has made it to the clinic. Other compounds, such as platensimycin and geldanamycin, have shown promise but either lack efficacy in humans or showed toxic side effects which prevented their development as drugs. South African researchers interested in natural products based drug discovery face the same challenges as elsewhere in the world. These challenges include the significant cost of drug development, re-isolation of previously reported compounds, and lack of interest in natural products for drug development. Nevertheless, the compounds reviewed here present only the tip of the iceberg and many more species remain to be discovered and studied for natural product production. Furthermore, with innovations and technological advancement in purification, structure elucidation, chemical biology, genome sequencing and mining, dereplication, and bioinformatic, cheminformatic and metabolomic tools like the GNPS molecular networking, microbial natural product drug discovery in South Africa shows great potential. It is worth mentioning that, apart from our research on the chemistry of the metabolites of the South African rare actinomycete Kribbella speibonae strain SK5, all the research on South African actinomycetes reported here was done by research...
groups based outside of South Africa. This represents a challenge and an opportunity for closer collaboration between South African researchers (microbiologists, pharmacologists and chemists) in order to fully explore the opportunities presented by South African microbial biodiversity.

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Competing interests
We have no competing interests to declare.

Authors’ contributions
K.S.A: Conceptualisation; data collection; writing – the initial draft. D.W.G: Conceptualisation; student supervision; writing – revisions. D.R.B: Conceptualisation; data collection; student supervision; writing – revisions.

Figure 10: Macrotermycins A–D 109–112 from South African actinomycetes Amycolatopsis sp. M39.

Figure 11: Secondary metabolites 113–122 from the South African Kribbella speibonae strain SK5.


