


REVIEW

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Uncovering the biodiversity and biosynthetic potentials of rare actinomycetes

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Abstract

Background: Antibiotic resistance is on the rise, and new antibiotic research has slowed in recent years, necessitating the discovery of possibly novel **microbial** resources capable of producing bioactive compounds. Microbial infections are gaining resistance to existing antibiotics, emphasizing the need for novel medicinal molecules to be discovered as soon as possible. Because the possibilities of isolating undiscovered actinomycetes strains have decreased, the quest for novel products has shifted to rare actinomycetes genera from regular environments or the identification of new species identified in unusual habitats.

Main body of the abstract: The non-streptomyces actinobacteria are known as rare actinomycetes that are extremely difficult to cultivate. Rare actinomycetes are known to produce a variety of secondary metabolites with varying medicinal value. In this review, we reported the diversity of rare actinomycetes in several habitat including soil, plants, aquatic environment, caves, insects and extreme environments. We also reported some isolation methods to easily recover rare Actinobacteria from various sources guided with some procedures to identify the rare Actinobacteria isolates. Finally, we reported the biosynthetic potential of rare actinomycetes and its role in the production of unique secondary metabolites that could be used in medicine, agriculture, and industry. These microbial resources will be of interest to humanity, as antibiotics, insecticides, anticancer, antioxidants, to mention but a few.

Short conclusion: Rare actinomycetes are increasingly being investigated for new medicinal compounds that could help to address existing human health challenges such as newly emerging infectious illnesses, antibiotic resistance, and metabolic disorders. The bioactive secondary metabolites from uncommon actinomycetes are the subject of this review, which focuses on their diversity in different habitats, isolation, identification and biosynthetic potentials.

Keywords: Rare actinomycetes, Bioprospecting, Biosynthetic genes, Genomic mining, Bioactive molecules

Background

Actinomycetes have long been recognized as a top source of biopharmaceuticals, particularly antibiotics [1, 2]. Gram-positive filamentous bacteria with a high G + C concentration are known as actinomycetes [3].

They are a key part of microbial diversity and have been found in a variety of habitats and unique settings. Rare actinomycetes are a group of actinomycetes whose isolation frequency is significantly lower than that of streptomyces strains obtained using traditional procedures [4]. Isolating and cultivating them is challenging. Due to their ability to produce a large variety of structurally diverse natural compounds with unusual bioactivity, these microbial groups from underexplored habitats are being studied in drug development [5]. They are found in a variety of habitats, including soil, aquatic, mangrove,

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desert, mountains, and plants, and account for around 10% of all isolated actinomycetes. They have shown to be an excellent and exciting source of novel and potent bioactive compounds [6]. Efforts in the past and present to isolate uncommon actinomycetes from underexplored diverse natural settings have resulted in the isolation of over 220 rare actinomycetes genera, with more than 50 taxa producing 2500 bioactive compounds [5]. This number accounts for more than a quarter of all actinomycetes metabolites, indicating that selective isolation techniques are being developed and widely used. This review updates all selected isolation medium, including pretreatment and enrichment procedures for the isolation of rare actinomycetes, to aid in that discovery. It reveals several processes toward the discovery of novel anti-microbial compounds from rare actinomycetes (Fig. 2). Furthermore, this research reveals that rigorous efforts in isolating and screening rare genera of actinomycetes from new and underexplored habitat can increase the discovery of new compounds with novel scaffolds. To address the rising number of antibiotic-resistant pathogenic bacteria, new antibiotics are minimally needed. Natural products continue to be the most potential source of new anti-microbials and bioactive compounds. Actinobacteria are well-known for being prolific makers of natural bioactive substances. Intensive efforts in isolating and screening rare genera of microorganisms are thought to boost the chances of identifying a new drug with a novel chemical structure. One strategy to break into novel bioactive chemical discovery is to screen rare actinomycetes and their hitherto underrepresented genera from unfamiliar settings in natural product screening collections [4]. The importance of unusual actinomycetes in this regard can also be shown in the fact that they produce several of the most effective antibacterial drugs now on the market. We want to refresh our understanding of the potential of rare actinomycetes by focusing on their biodiscovery potential; therefore, we want to give the reader a quick overview of the bioactive compounds from unusual actinomycetes. New compounds identified from these microbes with bioactive potential are the focus. Actinomycete strains that are difficult to identify are of particular interest to researchers. As a result, providing access to rare actinomycete strains with a high potential for producing novel bioactive compounds is of great importance [7].

The so-called "rare actinomycetes" are rather numerous in many habitats, according to molecular tools, and can be retrieved in large numbers using an appropriate isolation procedure [8]. We expect that investigating unusual actinomycetes that are difficult to isolate will yield a variety of beneficial compounds [9]. The distribution of rare actinomycetes is influenced by a variety

of parameters such as habitat type, ambient pH, and nutrient content [6]. The following genera are rare actinobacteria: *Gordonia*, *Isoptericola*, *Jiangella*, *Knoellia*, *Kocuria*, *Krasilnikoviella*, *Kribbella*, *Actinocorallia*, *Actinomadura*, *Agromyces*, *Alloactinosynnema*, *Amycolatopsis*, *Beutenbergia*, *Cellulosimicrobium*, *Gordonia*, *Isoptericola*, *Jiangella*, *Knoellia*, *Kocuria*, *Krasilnikoviella*, *Nocardia*, *Nocardioides*, *Nocardiosis*, *Nonomuraea*, *Oerskovia*, *Pseudokineococcus*, *Pseudonocardia*, *Rhodococcus*, *Saccharothrix*, *Streptosporangium*, and *Tsukamurella* [10].

It is challenging to isolate unusual actinomycetes using traditional dilution plate procedures. Isolation, preservation, and cultivation are all demanding procedures. The reason for this is that they are frequently obscured by fast-growing organisms including bacteria, fungus, and common *Streptomyces* [11]. Pretreatments such as dry heat, calcium carbonate, phenol, thermal, microwave, and sonication are required for the isolation of uncommon actinobacteria. One or more of these are done before plating the sample on appropriate media such as humic acid agar with vitamins (HVA) and oatmeal agar (ISP3), with 50 mg/L nalidixic acid and 100 mg/L of cycloheximide incubating at 30 °C for at least 7 days [12, 13]. These treatments remove non-filamentous bacteria from samples and restrict fungal growth, allowing slow-growing uncommon actinomycetes to thrive [12]. For fostering the growth of rare actinomycetes while suppressing bacterial and fungal contamination, appropriate selective media containing macromolecules such as casein, chitin, and humic acid are essential.

Diverse habitats for sourcing rare actinomycetes

Soil and plants

Actinomycetes populations have been thoroughly investigated in soil, and the majority of the rare actinomycetes reported so far have come from various types of soil [6]. Table 2 shows that the isolation of several novel and rare taxa mentioned in this analysis came from a variety of soil types. Many unusual actinomycetes are now being isolated from plants [14, 15], often to uncover new microbial resources for screening of potential bioactive compounds [16]. Endophytic habitats were used to isolate *Saccharopolyspora*, *Dietzia*, *Blastococcus*, *Dactylosporangium*, *Promicromonospora*, *Oerskovia*, *Actinocorallia*, and *Jiangella* species [17]. Endophytic Actinomycetes, such as the *Frankia* genera, can fix nitrogen, which is an important function in ecological systems [18]. Rare actinomycetes belonging to the *Micromonospora*, *Microbispora*, *Actinoplanes*, and *Streptosporangium* genera have been isolated consistently from numerous Korean soils [4].

Table 1 Different rare actinomycetes and their isolation media

S/N	New species of rare actinomycetes	Family	Sample source	Isolation medium	References
1	<i>Nocardioides mariniquilinus</i>	Nocardioideaceae	Coastal seawater	Marine agar	[35]
2	<i>Saccharomonospora amisosensis</i>	Pseudonocardiaceae	Deep marine	SM3 medium	[55]
3	<i>Nocardia</i> spp,	Nocardioideaceae	Zingiber officinale (root, stem)	Humic acid vitamin (HV) agar	[56]
4	<i>Saccharothrix xinjiiangensis</i>	Pseudonocardiaceae	Algerian soil	Chitin-Vitamin B medium supplemented with nalidixic acid and actidione	[57]
5	<i>Saccharomonospora oceani</i>	Pseudonocardiaceae	Marine sediment	Trypticase soy broth agar	[55]
6	<i>Nocardioides salsibiostraticola</i>	Nocardioideaceae	Sea water	R2A agar	[35]
7	<i>Micromonospora haikouensis</i>	Micromonosporaceae	Sanai desert of Egypt	Starch casein and Humic acid vitamin agar plates supplemented with cycloheximide	[1]
8	<i>Nocardioides rotundus</i>	Nocardioideaceae	Sea water	Modified ZoBell 2216E agar, ISP2 medium	[58]
9	<i>Verrucosipora andamanensis</i>	Micromonosporaceae	Marine sponge	Starch casein nitrate sea water agar	[59]
10	<i>Micromonospora spongicola</i>	Micromonosporaceae	Marine sponge	Starch casein nitrate agar	[59]
11	<i>Prauserella corallicola</i>	Pseudonocardiaceae	Marine coral <i>Galaxea fascicularis</i>	Yeast extract agar in 1L of sea water	[60]
12	<i>Saccharopolyspora spongiae</i>	Pseudonocardiaceae	<i>Scopalina ruetzleri</i>	M1 medium amended with cycloheximide and nystatin at 25 µg/mL each	[38]
13	<i>Microbacterium aureliae</i>	Microbacteriaceae	Moon jellyfish <i>Aurelia aurita</i>	Zobell marine agar and Tryptic soy agar	[61]
14	<i>Marmoricola aquaticus</i>	Nocardioideaceae	Marine sponge <i>Glodia corticostylifera</i>	M1 agar	[62]
15	<i>Arthrobacter echini</i>	Micrococcaceae	Purple sea <i>Heliocidaris crassispina</i>	Marine agar 2216	[63]
16	<i>Ornithinimicrobium algicola</i>	Intrasporangiaceae	<i>Ulva</i> sp.	Modified R2A medium	[64]
17	<i>Nocardia xestospongiae</i>	Nocardioideaceae	Marine sponge <i>Xestospongia</i> sp.	Modified starch casein nitrate sea water agar	[65]
18	<i>Rubrobacter aplysinae</i>	Rubrobacteraceae	<i>Aplysina aerophoba</i>	Tryptone soy agar	[66]
19	<i>Actinokineospora spheciospongiae</i>	Actinosynnemataceae	Marine sponge <i>Spheciospongia vagabunda</i>	ISP2 medium	[67]
20	<i>Williamsia spongiae</i>	Gordoniaceae	Marine sponge <i>Amphimedon viridis</i>	Tryptic soy agar	[37]
21	<i>Myceligenans cantabricum</i>	Promicromonosporaceae	Sea sediment	Tryptic soy agar supplemented with antifungal cycloheximide 80 µg/mL and nalidixic acid 20 mg/mL	[68]
22	<i>Saccharomonospora amisosensis</i>	Pseudonocardiaceae	Deep sea sediment	SM3 medium	[69]
23	<i>Saccharomonospora oceani</i>	Pseudonocardiaceae	Marine sediment	Tryptic soy broth agar	[55]
24	<i>Actinophytocola sediminis</i>	Pseudonocardiaceae	Marine sediment	Starch casein nitrate agar medium	[70]
25	<i>Pseudonocardia sediminis</i>	Pseudonocardiaceae	Sea sediment	DSMZ 621 medium	[71]
26	<i>Amycolatopsis flava</i>	Pseudonocardiaceae	Marine sediment	CMKA medium	[72]
27	<i>Saccharopolyspora griseoalba</i>	Pseudonocardiaceae	Marine sediment	CMKA medium	[40]
28	<i>Nocardioides litoris</i>	Nocardioideaceae	Beach sediment	Starch casein agar	[73]
29	<i>Streptomonospora nanhaiensis</i>	Nocardioideaceae	Marine sediment	Starch casein agar	[34]
30	<i>Agromyces marinus</i>	Microbacteriaceae	Marine sediment	NBRC medium	[74]
31	<i>Microbacterium enclense</i>	Microbacteriaceae	Marine sediment	Marine agar	[75]
32	<i>Microbacterium nanhaiense</i>	Microbacteriaceae	Sea sediment	Yeast extract-malt extract agar	[76]
33	<i>Glycomyces sambucus</i> sp. nov	Glycomycetaceae	Stem of <i>Sambucus adnata</i>	Humic acid vitamin agar	[77]
34	<i>Leifsonia ginseng</i> sp. nov	Actinobacteridae	Root of <i>Panax ginseng</i>	Humic acid vitamin agar, TWYNE agar	[44]

Table 1 (continued)

S/N	New species of rare actinomycetes	Family	Sample source	Isolation medium	References
35	<i>Glycomyces artemisiae</i> sp. nov	Glycomycetaceae	Root of <i>artemisia</i> sp. nov	Humic acid vitamin agar, Czapek's agar	[78]
36	<i>Pseudonocardia serianimatus</i> sp. nov	Pseudonocardiaceae	Leaves of <i>artemisia annua</i>	TWYE agar, Humic acid vitamin agar	[79]
37	<i>Pseudonocardia oroxyli</i> sp. nov	Pseudonocardiaceae	Root of <i>Oroxylum indicum</i>	Humic acid vitamin agar	[80]
38	<i>Zhihengliuella flava</i>	Micrococcaceae	Sea sediment	NBRC medium 802	[81]
39	<i>Kocuria indica</i>	Micrococcaceae	Marine sediment	Marine agar 2216	[82]
40	<i>Nesterenkonia alkaliphila</i>	Micrococcaceae	Deep sea sediment	Modified ISP1 agar	[83]
41	<i>Luteococcus sediminum</i>	Propionibacteriaceae	Sea floor sediment	Marine agar 2216	[84]
42	<i>Mariniluteococcus flavus</i>	Propionibacteriaceae	Deep sea sediment	HP agar medium	[85]
43	<i>Nocardia jiangsuensis</i>	Nocardiaceae	Costal sediment	Starch arginine agar	[86]
43	<i>Lysinimicrobium pelophilum</i>	Demequinaceae	Mud of mangrove	NBRC medium 802 supplemented with 5% w/v NaCl, 0.005% w/v cycloheximide and 0.002% w/v nalidixic acid	[87]
44	<i>Lysinimicrobium rhizosphaerae</i>	Demequinaceae	Soil of mangrove	NBRC medium 802 supplemented with 5% w/v NaCl, 0.005% w/v cycloheximide and 0.002% w/v nalidixic acid	[87]
45	<i>Micromonospora wenchangensis</i>	Micromonosporaceae	Mangrove soil	Glucose-peptone-tryptone agar supplemented with nystatin 50 mg/L, cycloheximide 50 mg/L, novobiocin 25 mg/L and nalidixic acid 20 mg/L	[88]
46	<i>Actinoallomurus acanthiterrae</i>	Thermomonosporaceae	Rhizosphere soil of <i>Acanthus ilicifolius</i>	Oatmeal agar supplemented with 25 µg/mL novobiocin, 30 µg/mL nystatin and 10 µg/mL nalidixic acid	[89]
47	<i>Ornithinimicrobium algicola</i>	Intrasporangiaceae	Green alga <i>Ulva</i> sp	Modified R2A medium	[64]
48	<i>Sinomonas humi</i>	Micrococcaceae	soil	Starch casein agar supplemented with cycloheximide 25 µg/mL and nystatin 10 µg/mL	[90]
49	<i>Nocardiopsis mangrovei</i>	Nocardiopsaceae	Mangrove sediment	Humic acid vitamin agar	[91]
50	<i>Kocuria pelophila</i>	Micrococcaceae	Rhizosphere soil of mangrove	NBRC medium 802	[92]
51	<i>Mumia flava</i>	Nocardioidaceae	soil	ISP2 medium supplemented with cycloheximide 25 µg/mL and nystatin 10 µg/mL	[93]
52	<i>Monashia flava</i>	Intrasporangiaceae	soil	Starch casein agar supplemented with cycloheximide 25 µg/mL and nystatin 10 µg/mL	[94]
53	<i>Kineococcus mangrovi</i>	Kineosporiaceae	Mangrove sediment	Starch casein agar supplemented with nalidixic acid 25 µg/mL and ketoconazole 100 µg/mL	[95]
54	<i>Nocardia</i> sp.	Nocardiaceae	Leaves <i>Zingiber officinale</i> plant	Humic acid vitamin agar (HVA)	[56]

Extreme environments

High and low temperatures, salt, alkaline and acidic pH, radioactivity, and high pressure are all examples of unique growth conditions found in extreme habitats. Microorganisms from harsh habitats have gotten a lot of attention because of their unique processes for adapting to their extreme surroundings and their ability to create uncommon bioactive compounds [19]. Despite the interest, actinomycetes that live in harsh settings have yet

to be extensively studied since the discovery of pioneer *Actinopolyspora halophila* by chance [5]. Researchers have been looking for unusual actinomycetes in a variety of habitats, including salt soil, alkaline soil, salty seas, and the ocean [20]. Researchers have isolated *Naxibacter*, *Actinopolyspora*, *Amycolatopsis*, *Citricoccus*, *Halomonas*, *Isoptericola*, *Jonesia*, *Kocuria*, *Kribbella*, *Liueella*, *Marinococcus*, *Massilia*, *Microbacterium*, *Nesterenkonia*, *Nocardia*, *Nocardiopsis*, *Prauserella*, *Rhodococcus*,

Saccharomonospora, *Saccharopolyspora*, *Sphingomona* from extreme environments [19]. Rare halophilic actinomycetes, such as *Nocardioopsis* strains, have been found to contain a high frequency of non-ribosomal peptide synthase (NRPS) genes, which could be linked to their great capacity for synthesizing huge numbers of physiologically active compounds [19].

Caves

Caves offer low nutrition, temperature, and light intensity as a microbiological environment, but high humidity [21]. These conditions may increase competition, which could boost the development of antibiotics and hydrolytic enzymes that stop other microbes from growing [22]. *Spirillospora*, *Nonomuraea*, *Catellatospora*, *Nonomuraea*, *Micromonospora*, isolated members of the *Actinomadura*, *Saccharopolyspora*, *Actinoplanes*, *Gordonia*, *Microbispora*, *Micromonospora*, *Nocardia*, and *Nonomuraea*, among others, have been isolated from caves. These findings support the idea that caves could be rich in rare actinomycetes that produce new compounds [22–26].

Insects and birds

The insect kingdom is yet another uncharted territory for discovering unique and unusual actinomycetes [27]. Some insects, such as *Pseudonocardia* and *Amycolatopsis*, kill weeds due to their natural ability to produce antimicrobials through a symbiotic interaction with actinomycete bacteria [28]. Insect-associated actinomycetes have been found to produce a few numbers of antifungal compounds. *Pseudonocardia* species isolated from lower attines *Apterostigma dentigerum* produced dentigerumycin, whereas *Streptomyces* species isolated from higher attine ants belonging to the genus *Acromyrmex* produced candicidin, a well-known antifungal [29, 30]. Antifungal activity was also observed in *Pseudonocardia* isolated from *Acromyrmex octospinosus*, although no antifungal compounds have been extracted or identified [29]. A *Pseudonocardia* species was recently discovered in the ant *Acromyrmex octospinosus* that produced a unique polyene antifungal metabolite [31]. Switching the search from explored to undiscovered areas could boost the discovery of new bioactive compounds [32]. *Streptosporangium*, *Actinomadura*, *Saccharopolyspora*, *Thermoactinomyces*, and *Nocardia* have recently been isolated from soils in the nests of solitary wasps and swallow birds [33]. Insects and birds are quickly becoming important sources for finding unique and novel bioactive compounds in Actinomycetes.

Aquatic habitat

In rivers, lakes, oceans, and marine habitats, rare actinomycetes are common. *Actinoplanes* with sporangium and zoospores will grow in moist environments and survive in dry environments as spores. *Micromonospora* spp. is a naturally occurring bacterium found in freshwater lakes and mud that can be isolated from lake sediments. Representatives of *Thermoactinomyces*, *Streptomyces*, and *Rhodococcus* have been found to be predominantly isolated from aquatic habitats, according to researchers [34]. *Actinoplanes*, *Actinomadura*, *Microbispora*, *Micropolyspora*, *Microtetraspora*, *Mycobacterium*, *Nocardioopsis*, *Nocardia*, *Promicromonospora*, *Rhodococcus*, *Saccharomonospora*, *Saccharopolyspora*, *Streptosporangium*, *Thermoactinomyces*, *Thermomonospora*, and *Thermopolyspora* are examples of rare genera of actinomycetes isolated from aquatic habitat [35].

Pretreatment of samples for isolation of rare actinomycetes

The discovery of humic acid vitamin agar (HVA) was a watershed moment in the isolation of uncommon actinomycetes. It is made entirely of soil humic acid, which is an excellent source of carbon and nitrogen for recovering rare actinomycetes from natural samples. Although humic acid is a highly heterogeneous cross-linked polymer that resists biological degradation and inhibits the formation of non-filamentous bacteria colonies, it is an exceptionally heterogeneous cross-linked polymer [4]. To limit duplication of isolation, different natural samples used for the isolation of unusual actinomycetes are frequently treated before the isolation to remove common actinomycetes like streptomyces and undesirable bacteria. For the isolation of rare actinomycetes from samples, a variety of pre-treatment methods and isolation media (Table 1) are used, including dilution and mixing with sterile natural decoction water from plant samples, seawater [36], artificial seawater, saline solution, and deionized/distilled water supplemented with NaCl for sea or marine sediment samples [37, 38]. A variety of pre-treatment procedures have been used to isolate uncommon actinomycetes selectively. Most researchers use drying and moist heating of sample materials [39], because actinomycetes spores are resistant to desiccation and heating, they can be used to screen against Gram-positive bacteria [39]. Because actinomycetes' spores are resistant to a variety of substances, including benzethonium chloride,

chlorhexidine gluconate, phenol, sodium dodecyl sulfate, and antibiotics, they are commonly used to isolate actinomycetes. These compounds can reduce or prevent the growth of aerobic Gram-negative bacteria, endospore-forming bacilli, and pseudomonads when treated with the samples for 30 min, improving the chances of isolating actinomycetes selectively [40]. The following sub-headings are used to discuss these pretreatment techniques:

Heat treatments

Most researchers propose using these pretreatment processes (wet and dry heat) in combination with selected isolation media for the selective isolation of novel and rare actinomycetes [4]. Most actinomycete genera' airborne spores are resistant to desiccation and have a significantly higher resilience to wet or dry heat than their vegetative hyphae [4]. The growth of *Streptosporangium* spp. is considerably aided by a dry heat treatment (120 °C for 1 h) of natural samples. Following surface sterilization and continuous drying at 100 °C for 15 min before directly plating on different selective media, numerous strains belonging to the genera *Pseudonocardia*, *Nocardiosis*, *Micromonospora*, *Microbispora*, *Actinomadura* and *Streptosporangium* were isolated [17]. Dry heating of samples treated with chemicals like 0.01 percent benzethonium chloride, 0.03 percent chlorhexidine gluconate, 0.05 percent sodium dodecylsulfate (SDS), 6 percent yeast extract, and 1.5 percent phenol and supplemented with different selective antibiotics like leucomycin and nalidixic acid on HVA has greatly increased the selectivity of rare actinomycetes [6, 41]. Pretreatment with moist (50 °C for 6 min) and dry (120 °C for 1 h) heating and 1.5 percent phenol reduced the quantity of unwanted bacteria and improved the selective separation of *Actinoplanes*, *Actinomadura*, *Saccharopolyspora*, *Gordonia*, *Microbispora*, *Micromonospora*, *Nocardia*, and *Nonomurea* [26].

Phenol treatment

Alternative approaches for the selective isolation of uncommon actinomycetes include adding chemicals such as phenol to natural samples [41]. Because 1.5 percent phenol is poisonous to bacteria, fungus, and streptomycetes, it increases the chances of isolating rare actinobacteria. As a result, 1.5 percent phenol treatment reduces the quantity of such organisms by removing sensitive species [42]. By pretreating samples with 1.5 percent phenol and then plating on HVA, several non-streptomycetes, including the rare genera *Actinomadura*, *Microbispora*, *Micromonospora*, *Nocardia*, *Polymorphospora*, and *Nonomurea*, were isolated [41, 43].

Selective antimicrobial agents

Several rare actinomycetes are resistant to a wide spectrum of antibiotics. Thus, several antibiotic molecules have been used in selective media to inhibit the competing bacteria including fast-growing actinomycetes. Selective isolation plates containing novobiocin significantly increased the numbers of *Micromonospora*-like colonies while gentamicin is also one of the selective agents used to access *Micromonospora* spp. [44]. Isolating media are mostly modified with nalidixic acid (50 mg liter⁻¹) and nystatin (100 mg liter⁻¹) to suppress the growth of Gram-negative bacteria and fungi [17].

Calcium carbonate treatment

The use of calcium carbonate to treat natural habitat samples enhanced the populations of rare actinomycetes genera [45]. Although the process is unknown, researchers discovered that mixing natural samples with calcium carbonate powder alters the pH in favor of actinomycete propagule growth, and the presence of calcium ions encourages the development of aerial mycelia in actinomycetes [46]. *Actinokineospora* spp., *Saccharopolyspora*, *Dietzia*, *Blastococcus*, *Dactylosporangium*, *Promicromonospora*, *Oerskovia*, *Actinocorallia*, and *Jiangella* species have all been successfully isolated using a combination of calcium carbonate rehydration and centrifugation [46, 47]. For the isolation of rare actinomycetes genera from natural samples, a combination of the calcium carbonate process and additional selective isolation procedures is usually recommended [45].

Microwave irradiation

The usage of microwave energy is commonly used to sterilize soil [48]. Total fungal and total prokaryote counts in soil extracts were lowered after microwave irradiation [49]. *Micromonospora*, *Micropolyspora*, *Nocardia*, *Actinomadura*, *Streptosporangium*, and *Lentzea* spp. are among the rare actinomycetes that have been isolated by microwave irradiation [48, 49]. Other physical agents are used to isolate rare actinomycetes in a selective manner. Electric pulses, electromagnetic radiation, super high frequency radiation, ultrasonic waves, and extremely high frequency radiation are some of the methods used [26, 50, 51]. The use of these techniques has resulted in a large rise in the overall number of isolated uncommon actinomycetes.

Centrifugation method

Another physical method is centrifugation, which removes Streptomycetes and other non-motile Actinomycetes from the liquid phase, allowing for the selective

growth of rare motile actinomycetes [46, 52]. Endophytic uncommon actinobacteria *Pseudonocardia*, *Nocardiopsis*, *Micromonospora*, *Amycolatopsis*, *Nocardia*, *Nonomuraea*, *Actinomadura*, *Gordonia*, *Promicromonospora*, and *Mycobacterium* species were isolated using a combination of enzymatic hydrolysis and differential centrifugation [53]

Chlorination and chemo-attractants

Selective isolation of sporulating actinomycetes known to produce motile spores can be done using xylose, chloride, γ -collidine, bromide and vanillin which act as chemo-attractants for accumulating spores of rare actinomycetes such as *Actinoplanes*, *Dactylosporangium* and *Catenuloplanes* [6]. The use of chloramine treatment has been used to selectively isolate rare genera *Herbidospora*, *Microbispora*, *Microtetrastora* and *Streptosporangium*. This is because chlorination is believed to suppress growth of contaminant bacteria but promote the growth of rare actinomycetes upon plating on humic acid vitamin media [6, 54]. Generally, rare actinomycetes are selectively isolated from natural habitats using combined physical and chemical treatments [45]. Several new Actinobacteria species are recovered from different sources using various media types (Table 1).

Isolation of rare actinomycetes

Collected samples (soil, marine sediment, plant parts) undergo series of pretreatments to promote the possibility of isolating rare actinomycetes and suppress the growth of often isolated streptomycetes [96]. These physical and chemical pretreatments include the use of dry heat, phenol treatments, sucrose gradient centrifugation and sodium dodecyl sulfate treatment [42, 97]. In case of isolating endophytic actinobacteria, plant samples are subjected to surface sterilization and are fragmented (8 × 8 mm) before deposition onto petri dishes containing the isolation media [98, 99]. Starch casein agar (SCA) and humic acid vitamin agar (HVA) supplemented with nalidixic acid (50 μ g/mL) and cycloheximide (100 μ g/mL) are mostly employed for selective isolation of rare actinomycetes [99]. The media are supplemented with a pinch of nalidixic and cycloheximide to inhibit unwanted bacterial and fungal contamination, respectively. An aliquot of 0.1 ml sample would be serially diluted up to 10^{-9} and a pour plate technique would be performed and incubated for 30 days at 28 °C and would be examined daily for the presence of colonies. The actinomycetes colonies are mostly identified by their chalky, powdery colonies and leathery texture [100]. These colonies would be sub-cultured

and maintained at 4 °C for further characterization. It is well established that several other antimicrobial agents such as anisomycin, gentamicin, kanamycin, novobiocin, nystatin, penicillin, primaricin, polymyxin, rifampicin, streptomycin, tunicamycin and vancomycin can also be added to the isolation media to promote the selective isolation of rare actinobacteria [54, 101].

Morphological identification of actinomycetes

Different culture media are employed to assess the macro-morphological characteristics of actinomycetes. These include: Agar yeast-malt extract (ISP2); Oatmeal Agar (ISP3); Agar Starch and inorganic salts (ISP4); Glycerol Asparagine Agar (ISP5), Soya bean meal agar, Glucose -Yeast Malt extract agar, Czapeks agar, Luria Bertani Agar (LBA), Starch casein agar and nutrient agar [102]. Each media would be sterilized, poured into sterile petri dishes and then left to solidify. Each strain would be aseptically streaked on the media surface and incubated at 28–30 °C for 7–21 days. The morphological characteristics to be examined among isolates include their color or soluble pigment, surface morphology, type of aerial hyphae, formation of aerial and substrate mycelia. These features are observed and compared using colour chart [102].

Microscopic characterization and biochemical tests for identification of actinomycetes

There are several microscopic and biochemical tests that are employed in identification of actinobacteria. They include Gram staining, starch hydrolysis test, casein hydrolysis test, urea hydrolysis test, lipase test, gelatin hydrolysis test, salt tolerance test, oxidase test, milk coagulation and peptonization test [103]. Most biochemical tests investigate the ability of the actinobacteria to produce different enzymes [104–106]. For example, coagulation and peptonization of milk test investigate the ability of the actinobacteria to produce protease enzyme, starch hydrolysis investigates their ability to produce certain exoenzymes like α -amylase and oligo-1,6-glucosidase while cellulose hydrolysis test checks the ability of actinobacteria to produce cellulase enzyme [107, 108].

Molecular and species level characterization

Sequel to morphological, microscopic and biochemical characterization, the isolated actinobacterial strains are subjected to species level identification done by 16S rRNA gene sequencing. The genomic DNA would be extracted using DNA extraction kit and the 16S rRNA gene amplified using pair of primers like (27F, 5'-AGA GTTTGATCMTGGCTCAG-3'; 1492R, 5'-GGTTAC CTTGTTACGACT T-3') and 9F(5'GAGTTTGATCCT

GGCTCAG3'); 1541R (5'AAGGAGGTGATCCAGCC3') [109, 110]. The amplified fragment for each strain would be sequenced utilizing the primers (forward and reverse). High-quality sequences would be assembled to produce the partial 16S rRNA contig for each strain. National Center for Biotechnology Information (NCBI) server are used to check the similarity for each contig against the available 16S rRNA genes data to determine the closest homologs. The homology search can be performed by comparing the sequence with thus present in the public database (NCBI) using the standard Basic Local Alignment Search Tool (BLAST) program. The 16S rRNA gene sequence of the selected strains would be submitted in the NCBI database to get GenBank accession numbers. For phylogenetic analysis, a neighbour joining tree based on the 16S rRNA gene sequences of the actinobacterial strains and their closely related type strains would be constructed at 1000 bootstrap replicates using by Molecular Evolutionary Genetic Analysis (MEGA) software [111, 112].

Genomic mining and omic based screening of rare actinomycetes

In rare actinomycete research, genome mining is an important bioprospecting tool. The fast advancement in genome sequencing, followed by mining of the genome using bioinformatic methods, including the identification of secondary metabolite gene clusters, has resulted in the finding of genetic machinery encoding for novel natural products from microbes that have yet to be chemically identified [113]. Polyketides (PK), non-ribosomally synthesized peptides (NRP), ribosomally and post-translationally modified peptides (RiPPs), and aminoglycosides are all encoded by most of these gene clusters [113]. Silent secondary metabolite gene clusters can also be discovered via bioinformatic analysis of genomes, which are not expressed under typical laboratory settings [114]. So far, more than 23,000 PK and NRP have been documented, many of which are discovered in actinomycetes and are being evaluated for pharmaceutical purposes [115, 116]. This method has also been utilized to discover novel antibiotic scaffolds in marine sediments from uncommon actinomycetes genera [117]. Due to revolutionary developments in genome- and metagenome-based approaches for drug discovery [118], the number of new biosynthetic gene clusters and corresponding compounds will undoubtedly increase in the near future, and it is likely that omics-based screening for novel bioactive compounds will overtake microbial isolation as the most efficient method for first identification of bioactive compounds [119].

The genes involved in the manufacture of bioactive secondary metabolites are found in the actinobacterial genome in the form of gene clusters, according to the literature [120]. Genome mining tools have made it more convenient to look for innovations in natural product discovery with majority of the bioactive compounds biosynthetic pathway of polyketides governed by a complex enzyme system, called polyketide synthase encoded by PKS gene cluster [121, 122]. Available whole genome draft of endophytic actinobacteria also revealed the presence of PKS and NPRS genes suggesting that these microbes are the possible source for many novel bioactive compounds [123, 124]. Screening for the presence of bioactive secondary metabolites in actinobacteria can be done using a high throughput method based on gene clusters. The antiSMASH (antibiotics & Secondary Metabolite Analysis Shell) pipeline is the first to identify biosynthetic loci across the whole spectrum of known secondary metabolite compound classes (polyketides, non-ribosomal peptides, terpenes, aminoglycosides, aminocoumarins, indolocarbazoles, antibiotics, bacteriocins, nucleosides, beta-lactams, butyrolactones, siderophores, melanins and others). It integrates or cross-links all previously existing secondary-metabolite specific gene analysis methods in one interactive view and aligns the detected regions at the gene cluster level to their nearest relatives from a database including all other known gene clusters [125].

Biopharmaceutical significance of rare actinomycete

Actinomycetes are major members of the soil microbial community, and their ability to create pharmaceutically useful compounds is of great interest to humans. Their interaction with rhizosphere soils has demonstrated their potential use as plant disease biocontrol agents. Their role as bioactive compound producers is well-documented. They are interesting prospects for the development of antimicrobials with medical and pharmaceutical applications [126].

Actinomycetes are known makers of antimicrobial compounds, which are significant medications in health care. Antibiotics could be produced by the genera *Streptomyces* and *Micromonospora* have shown to possess powerful therapeutic and acceptable pharmacokinetic qualities for clinical use [3]. Several substances derived from uncommon actinomycetes have been studied for their potential as antibacterial agents. Munumbicins were found to be efficient against *Mycobacterium tuberculosis* and *Bacillus anthracis* [127]. Actinomycetes produce

peptide antibiotics called kakadumycins, which have shown to be effective against *B. anthracis* [3]. Actinomycete-produced coronamycin was effective against pythiaceus fungi as well as the human pathogen *Cryptococcus neoformans* [128]. Maklamycin, an antibacterial polyketide discovered in the culture filtrate of *Micromonospora* isolated from the Thai medicinal plant *Abrus pulcellus*,

has been proven to be active against Gram-positive pathogens [129].

It is crucial to remember that biodiversity is the key to bioprospecting natural products. The isolation and discovery of new compounds with various chemical structures has frequently resulted from the diversity of microorganisms in unique habitats. When testing a molecule for a certain biological activity, multiple strains are

Table 2 Rare actinomycetes with their bioactive compounds

S/N	Bioactive compound	Chemical class	Source organism	Activity	Reference
1	Taromycin A	Lipopeptide	<i>Saccharomonospora</i> sp.	Anti-MDR pathogen	[147]
2	Retimycin A	Quinomycin-peptide	<i>Salinispora</i> sp	Cytotoxic	[148]
3	Sioxanthin	Carotenoid	<i>Salinispora</i> sp	Iron chelating	[149]
4	Lobosamide A-C	Polyene compound	<i>Micromonospora</i> sp	Anti-protozoan	[150]
5	Tetrocarcin N & O	Glycosides	<i>Micromonospora</i> sp.	Antibacterial	[151]
6	Nenestatin A	Benzofluorene	<i>Micromonospora echinospora</i>	Antibacterial	[152]
7	Thiasporines A-C	Thiazine	<i>Actinomycetospira chlora</i>	Cytotoxic	[153]
8	1,4-Dioxane	Dioxane	<i>Micromonospora</i> sp	Antibacterial	[154]
9	α -Pyrone 1–8	Pyrone	<i>Nocardiopsis</i> sp	Antibacterial	[154]
10	Glycerol 1-hydroxy-2,5-dimethyl benzoate	Salicylic derivative	<i>Verrucospora</i> sp	Anti-MRSA	[155]
11	Nocapyrones O-S	α -Pyrone	<i>Nocardiopsis</i> sp	Cytotoxicity	[155]
12	Nocazine F	Piperazine	<i>Nocardiopsis</i> sp	Cytotoxicity	[155]
13	Bramycin B	Macrolide	<i>Pseudonocardia carboxydivorans</i>	Antibacterial	[155]
14	Cyanogranide	Alkaloid	<i>Actinoalloteichus cyanogriseus</i>	MDR-reversing	[153]
15	Actinosporin A	O-glycosylated angucyclines	<i>Actinokineospora</i> sp	Anti-trypanosomal	[156]
16	Solwaric acids A & B	Aromatic acids	<i>Solwarospora</i> sp	Antibacterial	[156]
17	Seriniquinone	Quinones	<i>Serinicoccus</i> sp	Anticancer	[156]
18	Farozoline A	Polyketide	<i>Actinomadura</i> sp	Anti-candida	[156]
19	Amycolactam	Indole alkaloid	<i>Amycolatopsis</i> sp	Cytotoxic	[153]
20	Dermacozines H	Phenazine	<i>Dermacoccus abyssi</i>	Antioxidant	[156]
21	Microbacterins A & B	Peptaibols	<i>Microbacterium sediminis</i>	Cytotoxic	[154]
22	Salinipostins A-K	Phosphotriester	<i>Salinispora</i> sp	Anti-malaria	[154]
23	Saccharothrixones A-D	Aromatic polyketides	<i>Saccharothrix</i> sp	Cytotoxic	[154]
24	Telavancin	Glycopeptide	<i>Amycolatopsis orientalis</i>	Antimicrobial	[157]
25	Fidaxomicin	Tiacumicin	<i>Dactylosporangium aurantiacum</i>	Antimicrobial	[157]
26	Salinosporamide A	β -lactone- γ -lactam	<i>Salinispora tropica</i>	Anticancer	[158]
27	Arenamide A & B	Peptide	<i>Salinispora</i> sp	Anti-inflammatory	[159]
28	Anthracimycin	Polyketide	<i>Streptomyces</i> sp	Anti-anthrax	[160]
29	Halomadurone A	Pyrone	<i>Actinomadura</i> sp	Anti-proliferative	[156]
30	Levantilide C	Macrolides	<i>Micromonospora</i> sp	Anti-proliferative	[156]
31	Nocardiamide A, B	Hexapeptide	<i>Nicardiopsis</i> sp	Antimicrobial	[153]
32	Telithromycin	Macrolide	<i>Saccharopolyspora erythraea</i>	Antimicrobial	[157]
33	Biapenem	Carbapenem	<i>Streptomyces cattleya</i>	Antimicrobial	[157]
34	Ertapenem	Carbapenem	<i>Streptomyces cattleya</i>	Antibacterial	[157]
35	Daptomycin	Lipopeptide	<i>Streptomyces roseosporus</i>	Antibacterial	[157]
36	Tigecycline	Tetracycline	<i>Streptomyces aureofaciens</i>	Antimicrobial	[157]
37	Dalbavancin	Glycopeptide	<i>Nonomuria</i> sp	Antimicrobial	[157]
38	Oritavancin	Glycopeptide	<i>Amycolatopsis orientalis</i>	Antimicrobial	[157]
39	Tazobactam	β -lactamase inhibitor	<i>Actinomycete</i> sp	Antimicrobial	[157]

screened against a wide range of targets, and the positive result is referred to as the "lead." Deciphering the pathways involved in secondary metabolite production has proven valuable in determining a strain's metabolite-producing capacity. The polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) enzymes are encoded in the actinomycete genome. The ability of a strain to create secondary metabolites by the identification of these genes is reported using recognized primers [79]. This method eliminates the requirement to test many strains' fermentation products for bioactivities. The positive strains should be subjected to the metabolite-producing potentials in either case, as some of the genes encoding these pathways may not be functional or necessitating different growth conditions [15]. Bioactivities of several secondary metabolites isolated from uncommon actinomycetes have been examined, including:

Antimicrobial effect

Antibacterial activity of actinomycetes strains was significant and varied against Gram-negative and Gram-positive bacteria [130]. Because numerous bioactive compounds were secreted rather than a single inhibitory molecule, many actinomycetes possessed a diverse range of activities including antimicrobial activity [131]. Rare actinomycetes have been shown to have antifungal and antagonistic activities against human pathogens in recent decades [130]. Rare actinomycetes of the genera *Nocardia* and *Micromonospora* have been shown to be efficient against a variety of pathogenic yeasts, but the species *Nonomuraea* has shown only mild antibacterial action [132]. Furthermore, antimicrobial substances produced by uncommon actinomycetes of the genera *Micromonospora* and *Nocardia* had previously been discovered to have broad-spectrum activity against both bacterial and fungal infections [133, 134]. The emergence and spread of multi-resistant bacteria have affected practically all antimicrobial agent classes [135]. This necessitates a call for urgency in the quest for novel antimicrobials. Antimicrobial-resistant microorganisms have been identified as a serious global public health problem, resulting in increased morbidity, mortality, and healthcare costs [135]. Antibiotic misuse is frequent in many underdeveloped countries, resulting in large outbreaks of antimicrobial-resistant bacteria and a lack of surveillance and data collection. Antibiotics with novel structures derived from unusual actinomycetes are urgently needed to combat multidrug-resistant pathogenic bacteria. Natural products continue to be the best source of new antibiotics. Rare actinobacteria are known to be prolific producers of natural bioactive chemicals, hence, screening unusual actinomycetes isolates can be used for new antibiotic

discovery. We believe that intense efforts in isolating and screening rare genera of microbes can boost the chances of identifying a new drug with a novel chemical structure. One technique to do this is to screen rare actinomycetes and their previously under-represented taxa from unfamiliar settings in natural product screening collections [136]. Several bioactive substances derived from actinomycetes have been shown to suppress multidrug resistant pathogens such as vancomycin resistant *Enterococci*, methicillin resistant *Staphylococcus aureus*, *Shigella dysenteriae*, *Klebsiella* sp., *Escherichia coli*, and *Pseudomonas aeruginosa* [101, 137].

Antioxidant effect

To date, several actinobacterial antioxidants have been identified, including dihydroherbimycin A, N-carbamoyl-2,3-dihydroxybenzamide, 2-acetamido-3-(2,3-dihydroxybenzoylthio) propanoic acid, 2-allyloxyphenol, phenazines, and saccharomonopyrone A [138–142]. The genus *Streptomyces* has produced most physiologically active antioxidant compounds among actinobacteria [138]. Less prevalent or culturable strains of actinobacteria, such as rare genera, should be targeted for the discovery of new bioactive compounds due to the high likelihood of finding already known antioxidant metabolites (re-isolation of known antioxidant chemicals) [5]. UTM 537 *Saccharothrix ecbatanensis* is a valuable source for the development of multipotent antioxidant compounds [143].

Anticancer/cytotoxic effect

Despite major advancements in the treatment of malignant tumors, cancer remains a primary cause of death and a public health issue around the world. The prospect of microbial secondary metabolites represents an effective source for the development of therapeutic leads, among the keyways for the discovery of new bioactive molecules [144]. Many secondary metabolites from rare actinomycetes have been extracted and tested for anticancer activity in a variety of carcinoma cell lines, including K562 (Human acute myelocytic leukemia), HeLa (cervical carcinoma), AGS (Human gastric), MCF-7 (breast adenocarcinoma), and HL-60 (Human acute promyelocytic leukemia). The discovery of taxol, a strong anticancer agent derived from endophytic fungi, sparked an interest in microbes as a source of possible antitumor agents. The anticancer potentials of rare actinomycetes' staurosporine and kigamicin have also been investigated, with promising results [144].

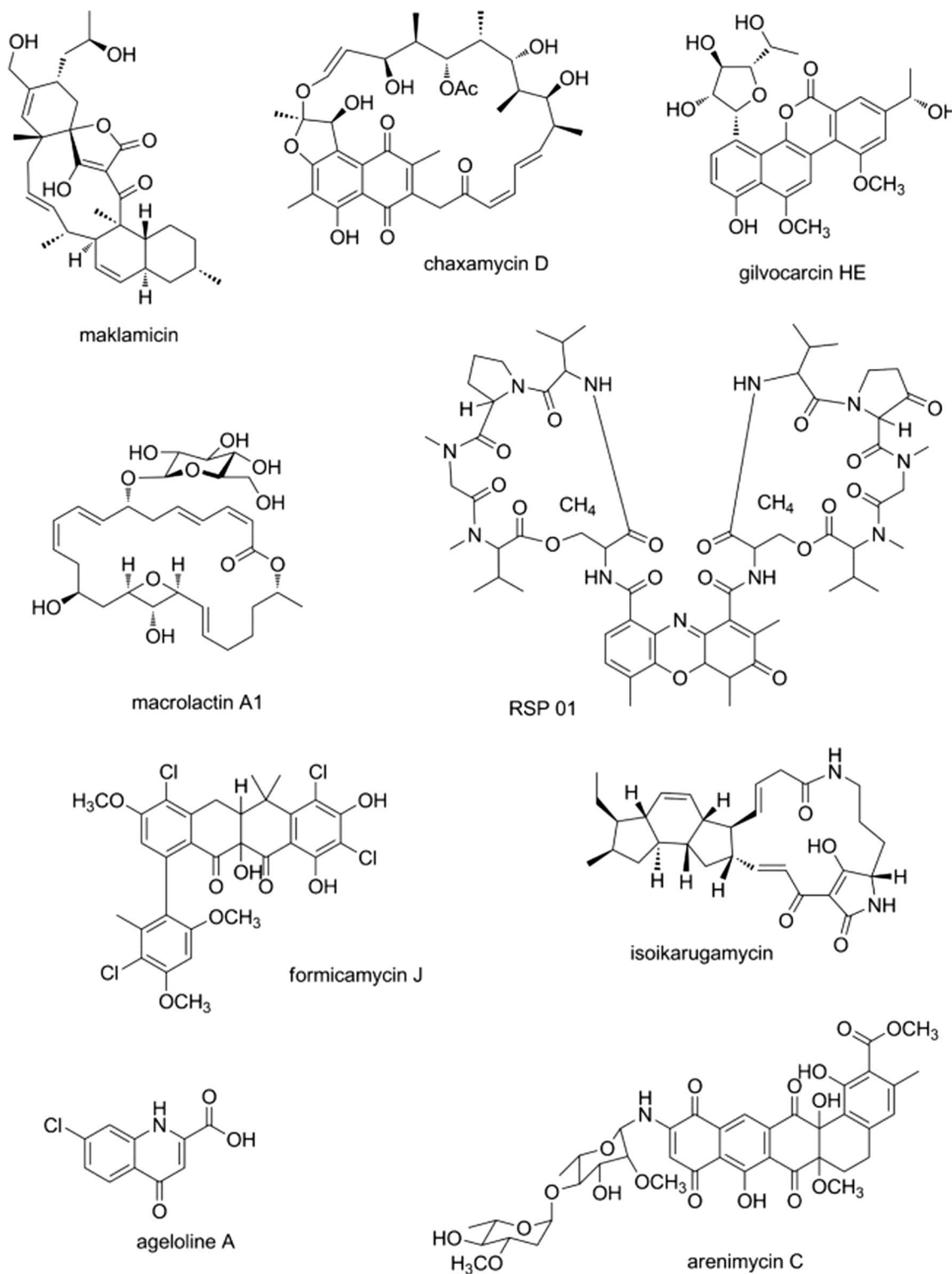


Fig. 1 Chemical structures of some bioactive compounds from rare actinomycetes

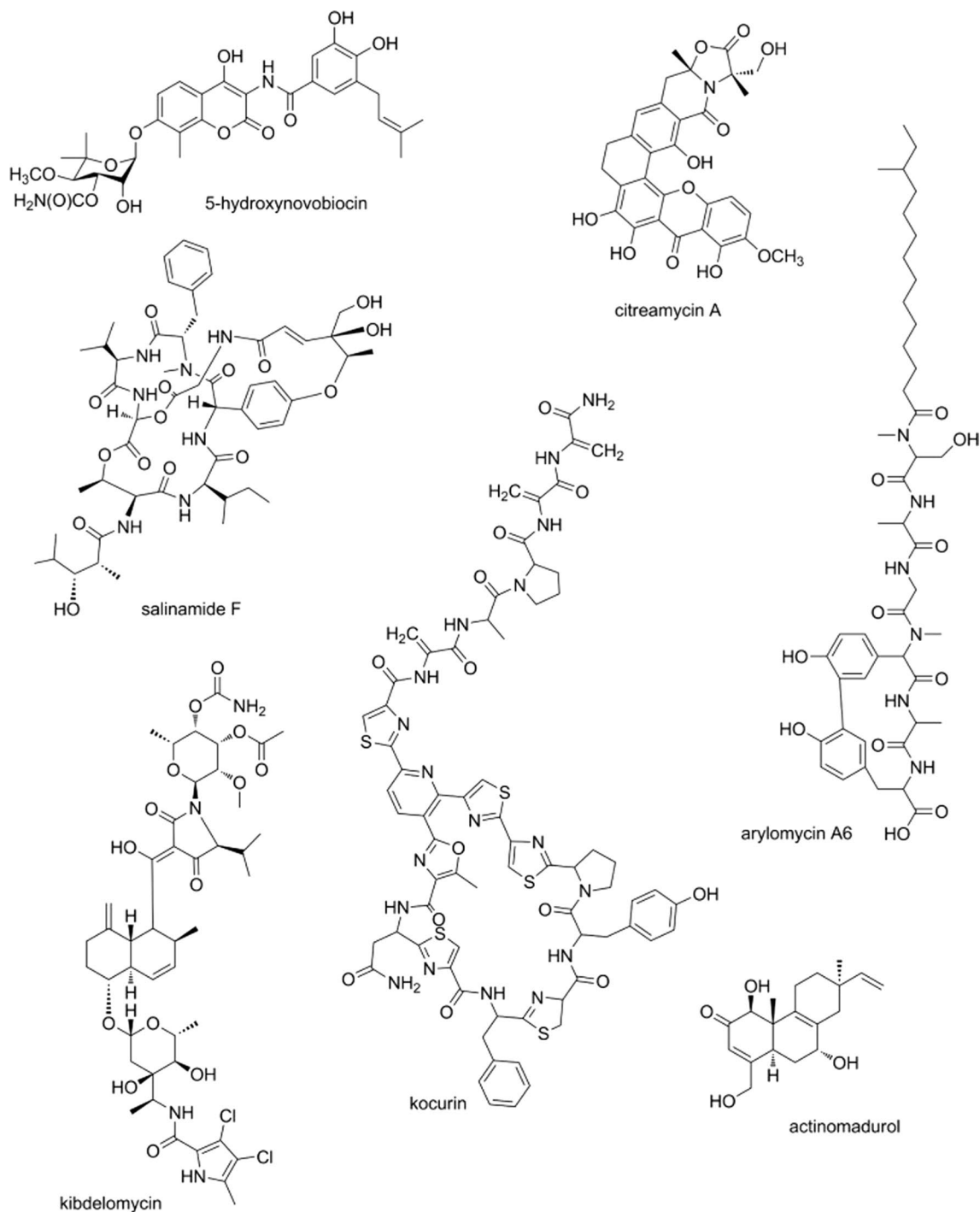
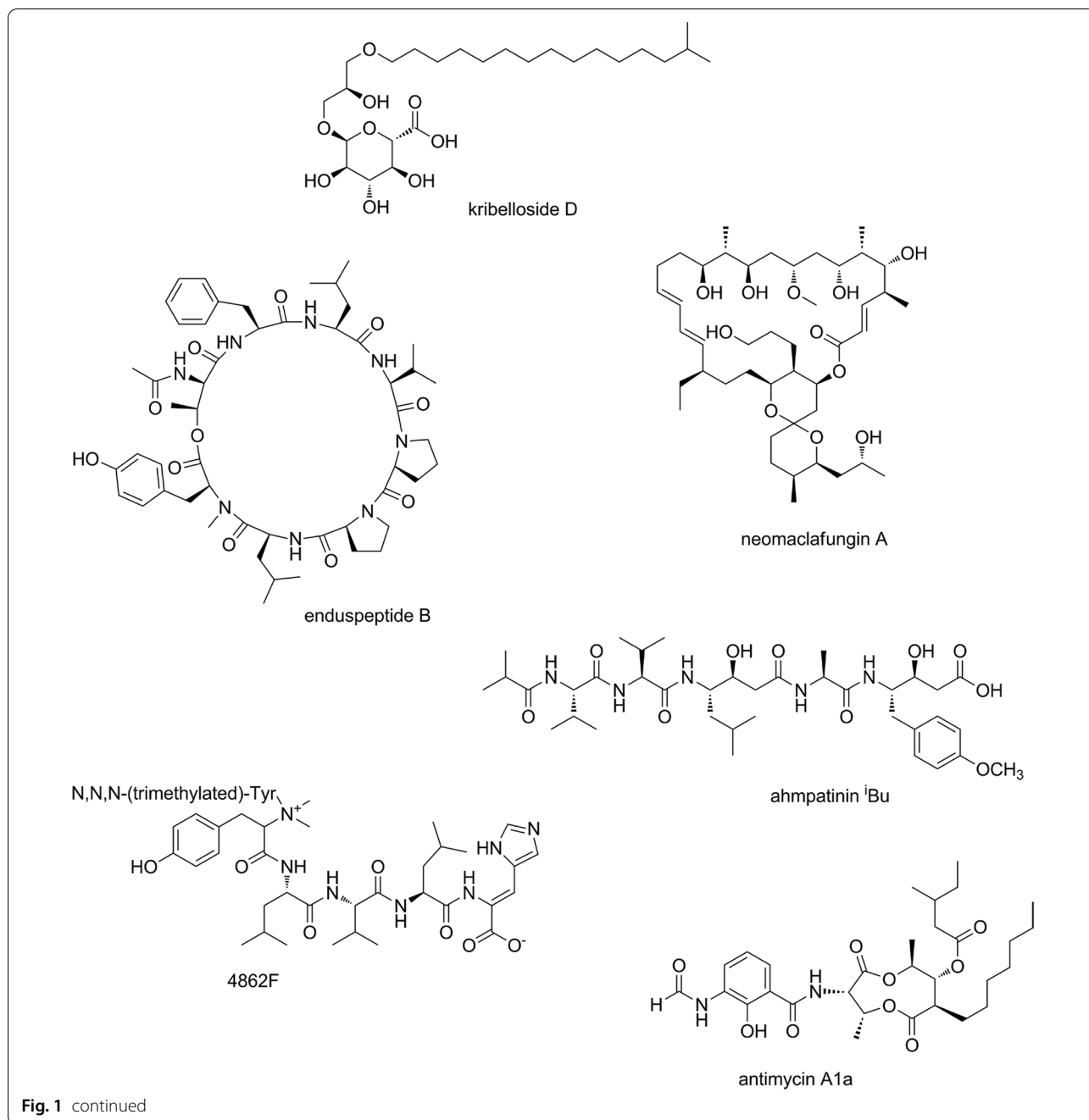


Fig. 1 continued

Insecticide/pesticide/herbicide

Pesticides made from natural products have grown in popularity around the world because to their excellent efficacy, environmental friendliness, and positive safety profile. This rise in popularity is reflected on the

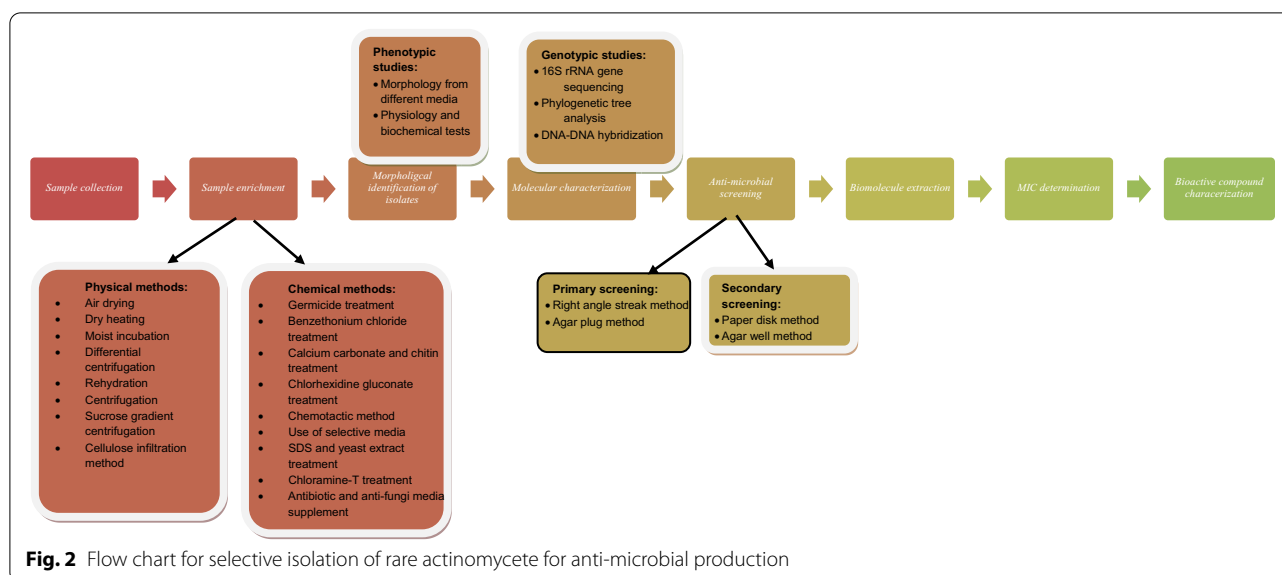
development of polyketide insecticides derived from actinomycetes in recent decades. Avermectins, spinosyns, polynactins, tetramycin, and analogues of these pesticides have all been used successfully in crop protection [145]. Furthermore, biotechnology's advancement



has resulted in ongoing improvements in the research and production procedures. *Actinomadura*, *Nocardioopsis*, *Dactylosporangium*, *Kibdelosporangium*, *Microbispora*, *Kitasatospora*, *Planomonospora*, *Planobispora*, *Salinispora*, *Marinispora*, *Serinicoccus*, and *Verrucosipora* are among the less well-known uncommon taxa. These consequences highlight the importance of continuing study in this domain, and investments in uncommon

actinomycetes can be deemed totally justified. PKS I, PKS II, and NRPS gene clusters were found in endophytic actinobacteria isolated from *Artemisia annua*, which had herbicidal activity against *Echinochloa crusgalli* [146]. Various antimicrobials and other bioactive compounds are obtained from rare actinomycetes (Table 2).

Several newer compounds isolated from rare actinomycetes include but not limited to Neomaclafungi A,



Maklamicin, chaxamycin D, Macrolactin AI, Gilvocarin HE, RSP 01, Formicamycin J, Isoikarugamycin, Ageloline A, Arenimycin C, 5-hydroxynovobiocin, citreamycin A, Salinamide F, Arylomycin A6, Kibdelomycin, Kocurin, actinomadurol, Kibdelomycin (Fig. 1). Neomacclafungi A is a metabolite product of *Actinoalloteichus* sp. with potent antimicrobial activity. Kibdelomycin is got from a rare actinomycete of genus *Kibdelosporangium*. Chaxamycin is a product of *Streptomyces* sp. strain C34. Maklamicin, salinamide F, Kocurin, actinomodurol, citreamycin A and Formicamycin J are respectively from *Actinomadura* sp TP-AO878, *Streptomyces* sp, *Kocuria palustris*, *Actinomadura* sp., *S. caelestis* and *S. formicae* [161–164].

Considerable factors affecting bioactive molecule production in rare actinomycetes

The ability of actinomycete cultures to form these bioactive products is not a fixed trait; it can be considerably enhanced or completely lost depending on nutrition and cultivating conditions [165, 166]. This is because antibiotic biosynthesis is a unique feature of bacteria that is highly dependent on growth conditions. Manipulation of the nutritional and physical characteristics of the culture environment can be used to improve growth and antibiotic production. As a result, media composition is critical to the efficiency and profitability of the final process. Therefore, choosing the right fermentation medium is crucial in the generation of secondary metabolites [165]. Antibiotic biosynthesis in actinomycetes has been shown to be affected by changes in the nature and type of carbon and nitrogen sources [167]. Several culture parameters like as pH, cell density, microbial strain, incubation time,

and temperature also play significant roles in the formation of bioactive metabolites [168]. When it comes to getting the best antibacterial output, cell density is crucial [169]. There are many natural products to be discovered from rare actinomycetes. Screening uncommon actinomycetes for novel bioactive metabolites is the first step in the search for useful antibiotics. This is followed by optimization of growth conditions for optimum antimicrobial compound production. Then comes antibiotic assay, chemical characterization, and identification of antibiotic compounds [101]. The amount and kind of actinomycetes present in the niche is influenced by ecological parameters such as environmental temperature and pH, habitat type, culture, organic matter concentration, exposure to air, and moisture content. Alkaliphilic actinomycetes, on the other hand, are extensively spread and easily isolated from their maritime environments [100, 169] (Fig. 2).

Conclusions

Rare actinomycetes have consistently produced a small number of novel bioactive compounds, but their promise in this field has been largely untapped. Due to the difficulty in cultivating most naturally occurring microorganisms, microbiologists have been severely limited in their research of natural microbial communities until recently. The search for unique biosynthetic potential species in unusual settings must be expanded. Microorganisms that are yet to be found or are rare may hold the key to developing new antibiotics to treat multidrug-resistant human infections and emerging fatal diseases. Using selective isolation and enhanced techniques, new rare bioactive producing actinobacteria can be discovered in previously unexplored environments. A combination of

pretreatment procedures, appropriate selective isolation media, and enrichment culture supplemented with specific antibiotics allowed the isolation of rare and unique actinomycetes that produced unusual bioactive compounds and new enzymes. Rare actinobacteria have new genomes and structural diversities that are just waiting to be identified and applied in biotechnological and pharmaceutical industries.

Abbreviations

ISP: International Streptomyces Project; HVA: Humic acid Vitamin Agar; LB: Luria Bertani Agar; SCA: Starch Casein Agar; NCBI: National Center for Biotechnology Information; BLAST: Basic Local Alignment Search Tool; rRNA: Ribosomal Ribonucleic Acid; DNA: Deoxyribonucleic Acid; MEGA: Molecular Evolutionary Genetic Analysis; MRSA: Methicillin Resistant *Staphylococcus aureus*; MDR: Multidrug Resistant; SDS: Sodium dodecylsulphate; PK: Polyketides; NRP: Non-ribosomally synthesized Peptides; RiPPs: Ribosomally and post-translationally modified peptides; PKS: Polyketide synthetase; NPRS: Non-ribosomal peptide synthetase; AntiSMASH: Antibiotic and Secondary Metabolite Analysis Shell.

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ECE conceived the project and was a major contributor in writing the manuscript; CFO helped in writing the manuscript. INH, DHA and MUE supervised the project. All authors read and approved the final manuscript.

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