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Review Article

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Rare Actinomycetes from Undiscovered Sources as a Source of Novel Antimicrobial Agents to Control Multidrug-Resistant Bacteria

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ABSTRACT

The major cause of death worldwide is infectious diseases and about 10 billion deaths, which represent 25% of all the major causes of death per year were recorded due to the rapidly increasing number of infections in hospitals among compromised and injured patients. Recently, the increasing prevalence of multi-drug resistant bacteria which cause serious healthcare-associated infections in hospitals were reported. The emergence of resistance to aminoglycosides, β lactam antibiotics, and methicillin-resistant Staphylococcus aureus cause serious risk for humans, and infections with resistant bacteria are difficult to treat. Vancomycin was earlier used to overcome the problem of methicillin-resistant S. aureus, but unfortunately, vancomycin-resistant S. aureus had appeared in hospitals. In addition, Pseudomonas aeruginosa, Acinetobacter baumannii, and Klebsiella pneumoniae received increasing attention as a determined as dangerous bacterial pathogens. Occurrence of penicillin-resistant Streptococcus pneumoniae is another resistant bacterium that cause infections in immunocompromised or old patients. As the rate of emergence of resistant isolates increased, the development of novel antibiotics is difficult. Actinomycetes, especially Streptomyces species are still the continuous sources of new products and more than two-thirds of the most used antibiotics are from actinomycetes. Streptomyces had the great biosynthetic ability to form antibiotics that stayed it without potential competition with other microbial genera. This review aimed to discuss the emergence of multidrug-resistant bacteria and their biocontrol with effective antimicrobial agents from rare actinomycetes. In conclusion, the development of novel antibiotics is still the aim of many studies, thus screening of new habitats for novel antibiotics is urgently needed.

Key words: *Multidrug-resistant, Streptomyces, Actinomycetes, Streptomycin gentamicin*

INTRODUCTION

In the world, antibiotic resistance pathogens are a serious health crisis and it is predicted to increase their numbers in the future. As new antibiotics are used, they face the rise of resistant pathogens after some time. The relatively high resistance of these pathogens to antibiotics depends on microbial origin and function in nature [1, 2]. In intensive care units, the sickest patients are infected with antibiotic-resistant bacteria, which are persistent in all communities and threaten human health worldwide [3]. Antibiotic overuse and the lack of new antibiotic development are causing this global crisis, which leads to the increasing spread of resistant bacteria worldwide. The development of new antibiotics to control antibiotic-resistant bacteria are greatly significant in new medicine to prevent and treat high-risk infections and increase survival rates [2]. With antibiotics, simple surgeries and small hurts become life threatening and take long time hospital stays. Medicinal chemist researchers efficiently tried to modify the previously discovered antibiotics or produce new products from novel strains from unexplored environments and focus on promising isolates. Their efforts lead to the development of the fourth and the third generation of β lactams and macrolides in addition to many other effective antibiotics [4]. The produced analogs of the previously used antibiotics may pose high inhibitory activity and decrease the existing bacterial resistance

but until now, there is a big shortage of effective safe antibiotics for MDR isolates of bacteria and fungi in hospitals [5]. This review aimed to discuss the increased microbial resistance and the use of secondary metabolites of rare actinomycetes from unexplored habituates to overcome and solve these problems.

MATERIALS AND METHODS

PubMed database was used for article selection, and the used keys words were Rare actinomycetes and their use in biotechnology OR actinomycetes uses in medicine. In regards to the inclusion criteria, the articles were selected based on the inclusion of one of the following topics: Rare actinomycetes, characters, and importance. Exclusion criteria were all other articles, which did not have one of these topics as their primary endpoint. About 97 publications were selected out of 1,200 articles indexed in the previous two decades, and their full texts were evaluated. A total of 53 of the 97 were included after a thorough examination. Additional research and publications were found using reference lists from the recognized and linked studies.

RESULTS AND DISCUSSION

Antibiotics and resistant problems

Antibiotics made an incredible contribution, perform an important role in several fields of modern society because they are valuable in industry, biotechnology, and pharmacology, and are mainly used to treat human microbial infections. They are secondary natural products produced from microorganisms and plants [6]. Antibiotic drug discovery depends on microbial secondary metabolites and natural products for production and developmental processes. Accordingly, the search for novel products has switched to rare genera of actinomycetes from normal habitats or to finding novel isolates in unusual habitats. The logic behind these ideas is that these isolates may produce new bioactive compounds. The unexplored and unexpected environmental habitats are the promising store of rare actinomycetes, rich with new and active antimicrobial agents [7].

In recent decades, isolation and exploitation of actinomycetes for novel compounds from conventional environments have led to the rediscovery of known compounds, thus pretreatment of isolation samples to get rid of the large numbers of gram-negative bacteria and allowing rare bacteria to grow. Different actinomycete isolates, obtained from soil, were screened for novel secondary metabolites, and a few relevant available commercially used antibiotics were deplored. Still, there are urgent orders to detect novel antimicrobial agents from actinomycetes to control the resistant problems of pathogenic microbes, which arise in hospitals worldwide [8]. In addition, there is several increasing need for more efficient enzymes in pharmaceutical industries [9, 10]. The subsequent development of antibiotics encouraged medicinal sectors and scientists to do their efforts to discover new microbial secondary metabolites for commercial antibiotic production and started the golden era of novel antibiotic discovery, which lead to the production of numerous life-saving antibiotics like streptomycin, vancomycin, and rifamycin, [11].

The antibiotic resistance problem is a big challenge, which threaded human life and the whole medical society however many infectious diseases re-emerged as a big risk to human life. In the clinical sector, the used antibiotics are developed from the secondary metabolites of microorganisms or derivatives of their products. The development of antibiotics has proceeded with the increase of microbes' resistant mechanisms, which are mainly associated with the presence of resistance genes. These resistant genes are found in the medium and easily be transferred to the microbe through many methods of gene transfer like transformation, conjunction, and transduction processes [12]. New antibiotics developments are not easy and are be considered labor-intensive methods and face many obstacles like the re-discovery of well-known compounds, low yields, high-cost compounds, and unsafe or unstable compounds. Therefore, our new strategy to discover new antibiotics must concentrate on new promising isolation sources, novel microorganisms with new chemical and bioactive secondary metabolites, and the use of new scientific methods for the purification and production of these antibiotics [12, 13].

Multidrug resistant Enterobacteriaceae

Multidrug-resistant Enterobacteriaceae is a worldwide urgent public health problem. Similar to other countries, Saudi Arabia is facing the challenge of increasingly reported cases of resistance [14], while Europe and the US have established monitoring programs for surveillance of antibiotic resistance, the rest of the world is still behind. Carbapenem resistance bacteria is a major and ongoing public health problem globally. It occurs mainly among Gram-negative pathogens such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa,* and *Acinetobacter*

baumannii, and may be intrinsic or mediated by transferable carbapenemase-encoding genes [15], The most effective carbapenemases, in terms of carbapenem hydrolysis and geographical spread, are the resistant genes of KPC, VIM, IMP, NDM and OXA-48 types [16].

Klebsiella pneumoniae is a prominent cause of nosocomial infections associated with high rates of morbidity and mortality, particularly in oncological patients [17]. *Klebsiella pneumoniae* is a major human pathogen with mortality rates up to 50%, particularly in immune-compromised individuals; it causes a broad spectrum of diseases including pneumonia, urinary tract infections, bloodstream infections, skin and soft tissue infections. Infections caused by carbapenem-resistant bacteria are extremely hard to treat and have been related to a high death rate [15] and turning into an inexorably troublesome issue in hospitals [18, 19].

Carbapenems are used to treat infections caused by bacteria resistance to β-lactams due to their broad spectrum of activity and stability. The high demand for carbapenems in clinical settings raises carbapenem resistance in Gram-negative bacteria including Enterobacterales, which considered a public health threat [20]. Infections caused by carbapenem-resistant bacteria are extremely hard to treat and have been related to a high death rate [21].

The continuous monitoring, molecular characterization, and identification of the source of the mechanisms of carbapenem resistance are required to limit the spread of antimicrobial resistance from becoming endemic in healthcare settings [22]. Bacteria normally were susceptible to different antibiotics but over time, they become resistant. Multidrug-resistant (MDR) which is identified as microorganisms that acquired resistance to at least one agent in three or more antimicrobial categories [23]. Infection prevention and control must be applied to prevent these bacteria to spread, It was identified as evidence-based practices and procedures that, when applied consistently in health care settings, can prevent or reduce the risk of transmission of microorganisms to health care providers, other clients, patients, residents and visitors.

Actinomycetes and their natural products

Actinomycetes are the heterogeneous filamentous or non-filamentous bacterial group with the prokaryotic nucleus and exhibit powdery growth, having aerial and substrate mycelia, sporangium, and chain of conidia. They are a diverse group of Gram-positive aerobic strict saprophytes bacteria with DNA content high in guanine and cytosine, and a G+C ratio was up to 80% [24]. Moreover, few isolates showed anaerobic respiration like *Actinomyces* or parasitic relationships with plants or animals [25]. They are abundantly found mainly in terrestrial and marine environments, in a symbiotic relationship with their plants and animals. They are one of the major microbial populations in soil; their numbers differ in different soil types, and marine water or association with marine animals [24]. They are identified as a group of bacteria with special characteristics and a magic ability to produce secondary metabolites [26]. Out of about 22,000 microbial secondary metabolites known, actinomycetes produce 70% of them followed by fungi (20%), and the genus *Bacillus* (7%) [7]. In general, they are the most economically and biotechnologically valuable bacteria with an excellent ability to produce many effective antibiotics and antitumor, immunosuppressive, antioxidant, and anti-inflammatory compounds.

Actinobacteria is a big phylum containing six classes, eighteen orders, sixty-three families, and up to forty, various genera like *Streptomyces, Saccharopolyspora, Amycolatopsis, Micromonospora,* and *Actinoplanes* [27]. The most important section of actinomycetes is called Streptomycetes, which contains the most important genus *Streptomyces* [26].

Rare Actinobacteria

Recently, big attention has been given to non-streptomycetes and rare actinomycetes found in undiscovered habitats [6]. It is well established that rare Actinobacteria are usually characterized as special genera of Actinobacteria rather than *Streptomyces* and their isolation frequency by conventional methods is rare or less than the strains of genus *Streptomyces*. Many studies reported the isolation of rare actinomycetes from different locations using special methods. Aly and El-Sabbagh, (2004) used different methods like wry heat, wet heat, special media, and the addition of phenol, antibiotics, or Calcium carbonate to the growth medium [28]. They isolated 50 rare actinomycetes from Nile river sediments with excellent activity against fungi, bacteria and cancer cells. They produce a wide variety of antibiotics. Members of rare genera, *Actinoplanes, Actinomadura, Microbispora, Micropolyspora, Microtetraspora, Mycobacterium, Nocardiopsis, Nocardia, Promicromonospora, Rhodococcus, Saccharomonospora, Saccharopolyspora, Streptosporangium, Thermoactinomyces, Thermomonospora,* and *Thermopolyspora* have also been reported from Chinese lake sediments [29]. Lynamicins is a group of antibiotics that was isolated from the rare actinomycete *Marinispora* sp.

and showed inhibitory activity against 11 bacterial pathogens of Gram-positive and negative bacteria, MRSA, and vancomycin-resistant *E. faecium*. In China, the rare actinomycete genera *Actinomadura, Isoptericola, Microbispora, Nocardia, Nonomuraea,* and *Rhodococcus* were isolated from mangrove soils and plants. Furthermore, for the first time, the rare actinomycete genus *Actinoalloteichus* was obtained from solar saltern [6]. Many bioactive compounds from members of the rare actinomycete genera like *Nonomuraea*, *Actinoalloteichus*, *Pseudonocardia*, *Saccharothrix*, and *Actinosynnema* were documented [6]. *Nocardiopsis alba* was isolated from mangrove soil and has a good ability to produce a bioactive compound with antioxidant properties. Mangrove sediments in Brazil were rich with rare actinomycetes where 14 new rare species, belonging to seven different families, have been characterized [7]. They belong to the genera *Brevibacterium, Dermabacter, Kytococcus, Microbacterium, Nesterenkonia,* and *Rothia*. It is reported that marine sediment is the best marine sample for rare actinomycete isolation and sediment collected from 5000 m depth in the Atlantic Ocean contains some rare actinomycete isolates [25]. A novel aerobic rare bacterium, *Streptomyces mangrovi* sp. nov, and another isolate with 93% similarity with *S. albogriseolus* NRRL B-1305 were isolated from a mangrove soil sample collected in Haikou, China and the two isolates possess good antimicrobial and antioxidant activity [30]. Bahamdain *et al*. (2020) isolated and molecular characterized some rare actinomycetes obtained from the Al-Lith Hot Spring Area of Saudi Arabia [31]. Most of the isolates showed moderate activity against some human bacterial pathogens.

Some rare actinomycetes have been isolated in unexplored extreme habitats**,** including arid areas, caves, hot springs, sandy clay soil, and marine ecosystems. They were studied as a big factor in many useful products from the marine water and the obtained isolates belong to genera *Nocardiopsis, Micromonospora, Salinispora,* and *Pseudonocardia* and produce many natural products and unique bioactive antibiotics [27]. The xinghaiamine A was extracted from the culture broth of the marine actinomycete *Streptomyces xinghaiensis* and exhibited broad-spectrum antibacterial activities against *Acinetobacter baumannii* and *S. aureus* and a potent cytotoxic activity against human cancer cell lines [30]. Aly *et al*. (2019) in a review reported the unexplored extreme habitats as sources of novel and rare actinomycete isolates with antimicrobial activities [32]. Isolation and molecular identification of *Streptomyces griseorubens* from Saman Region Cave, Saudi Arabia was carried out by Aly *et al*. (2020) [33]. The previous isolate showed good activity of the isolated actinomycetes against some multidrug-resistant bacterial pathogens.

Important secondary metabolites produced by actinomycetes

Actinomycetes produce several volatile substances like geosmin and methyl iso-borneol responsible for the characteristic "wet earth odor" [25]. Actinomycetes are the source of the most clinically used antibiotics and they produce approximately two-thirds of more than 10,000 known antibiotics. Each actinomycete strain has the genetic ability to produce many secondary metabolites and up to 20 effective materials [34]. These products of Actinomycetes can be used as antibiotics, antitumor agents, immune modifiers, and antitubercular disease [7]. **Table 1** showed the clinically and commercially useful antibiotics produced from the genus *Streptomyces* and rare genera of actinomycetes. Since the discovery of streptomycin from the strains of *Streptomyces griseus*, the greatest number of antibiotics introduced into the market were discovered from Actinobacteria like carbapenems (Cephalosporin), macrolides (Erythromycin), ansamycins (Rifampicin), glycopeptides (Vancomycin), and Tetracyclines (Demelocyclin) as reported by Mohammadipanah and Wink (2016) [35]. Drug discovery studied lead to the production of some commercially important antibiotics from the genus *Streptomyces* as daptomycin, lincomycin, isocoumarins, streptorubin B, pyrrole-2-carboxamide, acetyltryptamine, fervenulin and neomycin [11]. Other important antitumor drugs like anthracyclines (aclarubicin, daunomycin, and doxorubicin), peptides (bleomycin and actinomycin D), aureolic acids (mithramycin), enediynes (neocarzinostatin), antimetabolites (pentostatin), carzinophilin, and mitomycins were produced from other genera of Actinomycetes [34].

Table 1. Secondary metabolites produced by *Streptomyces* and rare actinomycetes

isolate	Active material	Active isolate	Activity	References
0ld Streptomyces genera	Axenomycins Aminoglycosides Dunaimycins Amphotericin B Cyclo (L-leucyl-l-prolyl) Chloramphenicol Tetracycline Musacin C	*Streptomyces lisandri* *Streptomyces diastatochromogenes* *Streptomyces nodosus* *Streptomyces* sp. KH614 *Streptomyces venezuelae* *Streptomyces aureofaciens* *Streptomyces griseovirdis*	Antibacterial	[36] [37] [38-40]

Recent Streptomyces genera	Asenjonamides A–C	*Streptomyces asenjonii*	Antibacterial	[41]
	Actinomycins V, X, and D	*Streptomyces antibioticus*	Antibacterial	[42]
	UND	*Streptomyces thermolilacinus*	Antibacterial	[43]
	Lucensomycin	*Streptomyces plumbeus*	Antifungal	[44]
Other Streptomyces genera	Gentamicin	*Micromonospora purpurea* var. *violaceae*	Antibacterial	[45]
	Rifamycin	*Amycolatopsis mediterranei*	Antibacterial	[46]
	Erythromycin	*Saccharopolyspora erythraea*	Antibacterial	[47]
	Lassomycin	*Lentzea kentukyensis*	Antituberculr	[48]
	Difluostatin A	*Micromonospora rosaria*	Antibacterial	[49]
	Salinosporamide A	*Salinispora tropica*	Anticancer, Antimalarial	[50]
	Atrop-abyssomicin C and proximicin A	*Verrucosispora maris*	Antitubercular, Antitumor	[51]

Actinomycetes from soil

Actinomycetes were highly spread in nature, in both terrestrial and aquatic ecosystems where they form a great part of the soil and live as free-living saprophytic bacteria. Some isolates can live inside the tissues of plants, insects, or aquatic animals. Few others can infect plants or animals causing mild diseases [52]. Many actinomycetes were obtained from alkaline soil, desert soil, and saltpan sand under the snow caps [9]. Many interesting actinobacteria were isolates in Egyptian soil and they showed inhibitory activity against many bacterial pathogens, *S. aureus*, *B. cereus*, *E. coli*, *K. pneumoniae*, *P. aerginosa*, *S. typhi* [53]. Also, Jeffrey (2008) [54] isolated 62 actinomycete isolates from the soil of Sarawak while *Streptomyces atrovirens* was isolated from the soil of Korea [55] while the isolated actinomycetes from different biotopes of Punjab, India showed a broad spectrum of antimicrobial activity [26]. Furthermore, 26 actinomycete isolates from Domang, India were obtaind, 12 (47%) of them had antifungal activity while 6 (23%) has antibacterial activity [56]. In addition, the three-isolated Actinobacteria showed broad-spectrum activity against Gram-positive and Gram-negative bacteria obtained from a soil sample, collected from Mount Everest [57]. Fatima *et al*., (2019) isolated 110 actinobacterial strains from the Pakistan desert and some of them exhibited promising antimicrobial activity against MRSA [58].

Actinomycetes from aquatic environments

Actinomycetes genera can inhabit aquatic environments and their counts depend on temperature, salinity, pH, and nutrient content. Actinomycetes are common in rivers, lakes, sea, and other marine environments and perhaps they are transported to the water from soil [59]. The presence of *Micromonospora* was documented in streams, rivers, and lake sediments [25]. Xu and Jiang (1996) studied the presence of actinomycete populations in 12 Chinese lakes and found *Micromonospora* (39-89%) the most dominant Actinomycetes followed by *Streptomyces,* which was the second most abundant genus [29]. Another study revealed the presence of some inhibitory actinomycetes for Gram-positive and negative bacteria in Fetzara Lake [60]). Poornima *et al*. (2008) isolated potential actinomycetes from shrimp pond sediments [61]. Also, Dias *et al*. (2009) isolated 238 bacterial isolates including actinomycetes from different sampling stations in mangrove regions of Ilha do Cardoso-Cananeia, Brazil, and reported that isolates of actinomycetes produced industrially important enzymes as amylase, lipase, esterase, and protease [62]. Raja *et al*. (2010) isolated 17 marine Actinobacteria strains from the rhizosphere sediments of mangroves and reported that the isolates SSR-2, SSR-3, and SSR-10 are potential inhibitors of many pathogens [63]. Furthermore, Girão *et al*. (2019) isolated 90 strains of actinomycetes from the macroalgae *Laminaria ochroleuca* and half of the isolates inhibited the growth of *C. albicans* and *S. aureus* and only one isolate showed anticancer activity against two human cancer cell lines [64].

Extreme environments as a source of actinomycetes

Extreme habitats are characterized by chemical or physical conditions that differ significantly from those found in environments that support more growth of large numbers of living organisms [65]. All extreme environments contain a variety of Actinomycetes that adapted themselves to grow in different ecological habitats like low

temperatures in glaciers, the deep sea, acidic and alkaline pH, industrial waste wastewater, high radiation area, high salt deserts and water, marine lakes, high temperatures places and in hot springs region. The obtained isolates from extremely habituate produced bioactive metabolites have exhibited relatively high thermal stability, bioavailability, and solubility [66].

Strains of *Streptomyces*, *Micromonospora*, *Saccharothrix*, *Streptosporangium*, and *Cellulomonas* were obtained from the Qinghai-Tibet Plateau [67], while *Micromonospora*, *Actinomadura*, and *Nocardiopsis* were reported from soda saline soils of the ephemeral salty lakes in Buryatiya [68]. Extremely halophilic filamentous actinomycetes were isolated from a hypersaline soil sample collected from the Jeddah region in the west of Saudi Arabia. In addition, high salt concentration and alkaline pH lakes are sources of novel actinomycetes that belong to the dominant *Streptomyces* genus. A novel halophilic actinomycete, *Nocardiopsis terrae* was isolated from saline water and produced the excellent antimicrobial agent, quinoline while Zhao *et al*. (2011) characterized actinopolysporins A, B, and C, new linear polyketides with antineoplastic activity from *Actinopolyspora erythraea* [69].

Arid regions contain unexplored actinobacteria with extreme habitat and can live in xerophilic, thermophilic, halophilic, and alkaliphilic habitats, producing new classes of antibiotics metabolites [35, 66]. At high temperatures or in hot spring areas, thermophilic actinomycetes were found in high abundances like species of the genera *Streptomyces*, *Micromonospora*, *Actinomadura*, and *Streptosporangium*. Ectoin and Hydroxyectoin with potential applications in industry and agriculture have also been identified from xerophilic actinobacteria [35]. Samples of the cave and cave-related habitats were used for the isolation of 47 species in 30 genera of Actinobacteria [10] while Zhang *et al*. (2018) obtained *Nocardioides allogilvus* sp. nov., from a soil sample collected from a karst cave in China [70]. Similarly, the antibiotic Xiakemycin A was produced from *Streptomyces* sp. from remote karst soil in China [67]. Although the low-temperature area considered the limiting factor affecting bacterial growth, *Streptomyces polaris* sp. nov. and *S septentrionalis* sp. nov. were isolated from a frozen soil sample which was collected from the Arctic region. Similarly, volcanic and geothermal areas with very high temperatures sometimes reached 70℃, some actinomycetes [9]. Some microbiologically diverse and specialized habitats for the isolation of thermophilic actinomycetes are desert soil, hot springs, volcanic eruptions, and thermal industrial wastes [31, 71].

Selective isolation of rare actinomycetes

Actinomycetes had slow growth compared to other soil bacteria [25]. Gram-negative bacteria inhibit the growth of some rare actinomycetes, which need selective isolation methods [72]. The use of specific nutritional media, the addition of some inhibitors like antibiotics to the growth medium, and the pretreatment of the soil samples with a physical or chemical method to decrease the number of other microorganisms and fungi increase the ability of rare Actinobacteria to appear and grow. The isolation frequency of rare actinomycetes is much lower than that of the Streptomyces strains isolated by conventional methods.

Pretreatment, both chemical and physical methods are generally useful for the isolation of various actinobacterial species [10]. Actinobacteria can be easily isolated by physical pretreatment methods, but to isolate other than streptomycetes, chemical or combinations of physical and chemical methods are used [70]. Physical pretreatments like air-drying, moist heat, dry heat, and electromagnetic wave can be used to increase the ratio of actinomycetes isolation [10, 70]. Air-drying of soil at 120℃ for one-hour is usually preferred for the isolation of genera *Dactylosporangium*, *Microbispora*, and *Streptosporangium* [67]. *Micromonospora* were selectively isolated by pretreatment of samples at 55-65℃ for 30 min. In another study, soil samples dried at 45℃ for 1 hr. in a hot air oven were used for the isolation of different genera of rare Actinobacteria while moist heating was useful for eliminating fast-growing bacteria and allowing the growth of rare actinomycetes [73].

Chemicals treatments such as the addition of calcium carbonate, chitin, calcium chloride, sodium chloride, phenol, SDS, and chemotactic agents enhanced the isolation of rare actinomycete genera [10, 72, 74]. Phenol pretreatment is used for the selective isolation of *Micromonospora* (49.18%), *Streptomyces*, *Actinomadura*, *Microbispora*, and *Polymorphospora*. Fang *et al*. (2017) reported that pretreated the samples collected from Sigangli Cave, China using heat, different pHs, and supplementation of media with different calcium salts help the isolation process of rare genera of Actinobacteria [74]. *Actinomadura* and *Saccharopolyspora* were two rare genera isolated from a caves soil sample, pretreated with heat and using a selective isolation medium [33]. Samples collected from the Cholistan desert, Pakistan was heat treated at 50-55℃ for 2-16 hrs and chemically treated with $CaCO_3$ (10:1 w/w) and used for the selection of rare Actinobacteria [58].

CONCLUSION

Actinomycetes or filamentous bacteria had a prokaryotic nucleus powdery growth, aerial and substrate mycelia, sporangium, and chain of conidia. They belong to Gram-positive aerobic bacteria with DNA content up to 80%. They are found mainly in soil and marine environments or in association with some plants and animals. They are identified as a group of bacteria with special characteristics and a magic ability to produce secondary metabolites like antibiotics and antitumor, immunosuppressive, antioxidant, and anti-inflammatory compounds.

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