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Actinomycetes: Dependable Sources for Industrially Useful Secondary Metabolites

Olusegun Richard Adeoyo

Microbiology Department, Adekunle Ajasin University, P.M.B. 001, Akungba-Akoko, Ondo State, Nigeria.

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Abstract: **Actinomycetes are saphrophytic, free-living bacteria that are abundant in soil, water, and plant roots. They belong to one of the major bacterial phyla and are Gram-positive bacteria with G+C DNA. They survive in settings of moderate to complex environments. This review aimed at highlighting the potential of actinomycetes as reliable sources of biomolecules that are valuable for industries. These bacteria produce beneficial secondary metabolites like enzymes (amylase, protease, lipase, cellulase, xylanase, and chitinase), antioxidants, and antimicrobials that are industrially useful. Therefore, this review underpins the use of various actinomycetes secondary metabolites in a variety of industries that include pharmaceutical, textile, food, paper, detergent, and agriculture.**

***Keywords*: Actinobacteria, bioactive compounds, Antimicrobials, Antioxidants, Microbial enzymes.**

1. INTRODUCTION

Actinomycetes (actinobacteria) are aerobic, sporulating, filamentous, and Gram positive group of bacteria of the order Actinomycetales characterized by substrate and aerial mycelium growth, with a high guanine plus cytosine (G + C) content of 57-75% in their DNA (Deepika and Kannabiran, 2010). They are one of the biggest phyla of bacteria, with 130 genera and 41 family members. According to Cruz *et al.* (2015), they exhibit a broad variety of various morphological traits, such as coccoid to rod-coccoid cell shape, aerial and substrate mycelia color, and fragmenting hyphal forms. The Greek words *atkis*, which means ray, and *mykes*, which means fungus, were combined to create the name Actinomycetes. They must have distinguishing characteristics to be classified as "Kingdom bacteria" (Chaudhary *et al.*, 2013). Actinomycetes are unicellular like bacteria, but they don't have distinct cell walls that lack chitin and cellulose, which are frequently seen in the cell walls of fungi. Instead, they create thin, non-septate mycelium like real bacteria do. Colonies of actinomycetes develop slowly, take on a powdery appearance, and adhere firmly to the surface of the culture media (solid). Like fungi, they also produce sporangia and hyphae (Cruz *et al.*, 2015; Chaudhary *et al.*, 2013).

Barka *et al.* (2015) reported that actinomycetes are primarily saprophytic soil dwellers. They can survive in a variety of ecological environments besides soil, such as fresh and salt water, and the air, but they are more prevalent there than in other ecological environments, particularly in alkaline soils and soils rich in organic matter (Barka *et al.*, 2015). They are an integral part of the microbial population. The process aids in the formation of soil humus as they recycle the nutrients associated with recalcitrant polymers, such as chitin, keratin, and lignocelluloses. They play a crucial role in recycling refractory biomaterials by decomposing complex mixtures of polymers in dead plants, animals, and fungal materials that they use as substrates for their metabolic activities (Barka *et al.*, 2015; Sharma *et al.*, 2014; Stach and Bull, 2005). Actinomycetes, which are fungi that develop as hyphae like filaments in the soil, are what give freshly churned, healthy soil its distinctively earthy odour.

According to Chaudhary *et al.* (2013), actinomycetes are distinguished by the production of typically branching threads or rods. Although the hyphae are typically non-septate, septa may occasionally be seen in some forms under specific circumstances. The mycelium that is producing spores might be straight or spiral in shape, branching or not branching. (Chaudhary *et al.*, 2013) The spores might be spherical, cylindrical, or oval. According to Chaudhary *et al.* (2013), actinomycetes create early microcolonies made up of branching system filaments that fragment into diphtheroid, short chain, and coccobacillary forms within 24 to 48 hours. The actinomycete cell wall is a hard structure that preserves the cell wall's shape and prevents the cell wall from breaking owing to excessive osmotic pressure.

The wall is made up of a wide range of complex substances, including peptidoglycan, teichoic and teichuronic acid, and polysaccharides (Barka *et al.*, 2015). The peptidoglycan, which is unique to prokaryotic cell walls, is made up of glycan (polysaccharides) chains of alternating N-acetyl-d-glucosamine (NAG), N-acetyl-d-muramic acid (NAM), and diaminopimelic acid (DAP). Chemical bonds hold teichoic and teichuronic acid to peptidoglycan. The chemical makeup of their cell walls is comparable to that of Gram positive bacteria, yet actinomycetes have been regarded as a distinct group from other common bacteria because of their well-developed morphological (hyphae) and cultural traits (Chamikara, 2016). Actinomycetes such as *Streptomyces* spp. from the root that is in touch with soil are always present on the surface of plants and occasionally even in different areas of the plants themselves. Only a small number belonged to *Streptoverticillium* genus, while the majority is *Streptomyces* species (Petrolini *et al.*, 1996).

Growth conditions are requirements either as presence/absence of oxygen and nitrogen or physical such as temperature and pH level that are essential for the successful growth of microorganisms. Actinomycetes are mostly encountered in dry alkaline soil but they are also able to withstand other extreme conditions such as high salinity. They produce spores as a means dispersal and also for survival under unfavorable conditions such as limited nutrient supply (Jeffrey, 2008; Barka *et al.*, 2015), like other bacteria, actinomycetes are predominantly mesophilic, with optimal growth temperature between 25-30ºC and certain species which are thermophilic, can grow between 50-60ºC (Barka *et al.*, 2015). Actinomycetes growth best at a pH 6-9, with maximum growth around neutrality, but some strains can survive under acidic conditions.

Reproduction of actinomycetes is usually asexual, though sexual processes have been shown to occur by genetic analysis. In non-hyphal forms, asexual reproduction is by fragmentation or by the usual fission of single cells. Vegetative reproduction is by well-formed spores resembling fungal arthrospores (Saiman *et al.*, 2004). *Streptomyces* and *Frankia* spp have a similar life cycle to that of fungi (Chaia *et al.*, 2010). *Streptomyces* species sporulation is believed to be linked to stresses that induce secondary metabolite production such as exhaustion of available nutrients. At this stage, the colony of bacteria forms aerial hyphae (Anderson and Wellington, 2001). Until the multi-genomic terminals of the hyphal growth complete mitosis-like segregation of bacterium chromosomes and plasmids to create uni-genomic spore compartments, continued aerial hyphale growth is maintained by using the vegetative mycelium (Sahin and Ugur, 2003). On solid media, *Streptomyces* spp. forms characteristics around the colonies with a depression in the middle. Aerial hyphae extend upwards and produce spore compartments when conditions are favorable for growth.

Actinomycete molecular categorization has its roots in early nucleic acid hybridization investigations but has evolved since the development of nucleic acid sequencing methods. In the systematics of bacteria and actinomycetes, the importance of phylogenetic analyses based on 16S rRNA sequences is rising. A phylogenetic tree based on the 16S ribosomal DNA sequences has been published, allowing for the research of the evolution of actinomycetes as well as serving as a basis for identification. Beginning with DNA isolation and polymerase chain reaction amplification of the 16S rRNA gene, 16S rRNA analysis is carried out (Sahin and Ugur, 2003). Typically, 16S rRNA enables for organism identification up to the genus level. The purpose of this review was to highlight the potential of actinomycetes as reliable sources of commercially viable bioactive compounds.

Secondary Metabolites Production

Secondary metabolites are compounds produced by a cell through the process of cellular metabolism. Their production is majorly influenced by environmental factors (such as nutrient depletion). They are produced at the stationary growth phase and not necessarily involved in cellular growth and reproduction (Anderson and Wellington, 2001). However they often give an organism a competitive advantage in a particular environment. Secondary metabolite production is often regulated and only initiated through environmental stress. Most soil bacteria e.g., actinomycetes often produce these biologically active secondary metabolites. Geosmin produced by *Streptomyces* species has an earthy aroma; it is responsible for the earthy taste of vegetables such as beets and adds to scent of disturbed soil (Anderson and Wellington, 2001).

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Actinomycetes in ecosystem

Actinomycetes are found in quite a significant number as a major component in most soils. They play a crucial role in the crucial role in the cycling of nutrients in soil ecosystems. They are involved in the decomposition of organic matter and the release of nutrients such as nitrogen, phosphorus and sulfur, which are essential for plant growth (Wang *et al.*, 2014). They are also capable of degrading a wide range of organic pollutants, including polycyclic aromatic hydrocarbons (PAHs), petroleum hydrocarbons, and pesticides. They produce various enzymes such as cellulases, lignin peroxidase, laccases, and xylanases, which are involved in the degradation of these pollutants.

Bentley *et al.* (2002) discussed about microbial metabolites production that filamentous actinomycetes produced over 10,000 bioactive compounds (45% of all microbial metabolites), 75% of valuable production were of *Streptomyces* spp. origin and 25% were from rare actinomycetes (*Micromonospora*, *Actinomadura* and *Streptoverticillium*. Among the actinomycetes, *Streptomyces* spp contributed the greatest chemical diversity. A study on obtaining antifungal compounds from marine *Streptomyces* spp was carried out by Cho *et al.*, (1999). These compounds displayed strong antifungal activity against *C. albicans*, *E.coli* and *P. aerogenosa*. Also, Ogunmwonyi *et al.* (2010) reported a wide range antimicrobial activity of ten most potent marine *Streptomyces* spp. isolated from the Nahoon beach, a coastal shore of Indian Ocean in the Eastern Cape Province of South Africa.

Actinomycetes as antimicrobial agents

The first antibiotic discovered by Sir Alexander Fleming in l928 (Fleming, l929) facilitated the discovery of many other secondary metabolites with similar properties. A lot of antimicrobial agents have been isolated from actinomycetes and are extremely useful in medicine (Hasani *et al.*, 2014; Wang *et al.*, 2020; Amin *et al.*, 2021). Among the actinomycetes, genus *Streptomyces* is established as a major source of microbially derived antibiotics (Boonlarppradab *et al.*, 2008). Many antibiotics use in medicine around the world come from actinobacteria. Studies have revealed that *Streptomyces* could be the source of new antibiotics needed to fight methicillin-resistant *Staphylococcus aureus* (MRSA) and other infections that are resistant to commonly-used medicines. This group of bacteria could also provide new antifungal and anti-parasitic drugs for people and livestock, as well as compounds used to fight cancer and during transplant operations (Bentley *et al.*, 2002).

Polyoxin is a secondary metabolite with a single-site mode of action. It is a fungicide that can be produced by *S. cacaoi* var. *asoensis* and active against phytopathogenic fungi such as *Alternaria kikuchiana* (a fungus of black spot disease) and *Pricularia oryzae* (a rice blast fungus) (Kimura and Bugg, 2003). Nikkomycin is another bioactive compound that can be produced by *S. tendae* and *S. ansochromogenes* (Kimura and Bugg, 2003) and function as inhibitors of chitin synthase in fungi (Liao *et al.*, 2009). It has been revealed to act against *Candida albicans* as well as *Rhizopus carcinans* and *Botrytis cinerea*) (Kimura and Bugg, 2003). Also, Amorim *et al.* (2020) reported that *Streptomyces ansochromogene* had antibacterial activity against *Pseudomonas aeruginosa* and posited that the metabolite produced is a promising bioactive agent against bacterial resistance. Additionally, *S. nanchangensis* can secrete insecticidal compounds such as nanchangmycin, meilingmycin and avermectin, they are active against harmful nematodes and insects (Sun *et al.*, 2003).

In rhizosphere soil, actinomycetes are capable of producing antibiotics and other useful metabolites. These antibiotics play an important role in the defense mechanism of soil microorganism and help to control the growth of other microorganisms in the ecosystem (Netzker, 2015). Therefore, they have possibility in influencing the rhizospheric pathogens. *Streptomyces* produce beta-lactam compounds that affect bacterial cell by inhibiting cell wall synthesis. This is done by interfering with mucopeptide synthesis thereby, resulting in the production of deficient cell walls. *Streptomyces clavuligerus* is a good producer of a variety of B-lactam compound including penicillin (Nathwani, 2005).

Actinomycetes as antioxidant sources

Antioxidants play an important role in inhibiting and scavenging free radicals, by so doing provide protection to humans against various infections and degenerative diseases (Sharma and Gupta, 2008). It should be noted that oxidative stress can occur when there is increased free radicals or decreased antioxidant. Response of *Streptomyces* growth to oxidative stress conditions had been examined in the past by Schweder *et al.* (2005). Similarly, a *Nocardiopsis alba* had been screened *in vitro* for antioxidant capacity where one of the fractions indicated the presence of ascorbic acid (Janardhan *et* *al.*, 2014). There are certain naturally occurring antioxidants that can give protection against oxidative stress induced damage in human cells. Modern research is now directed towards natural antioxidants from plants and microorganisms which serve as safe therapeutics (Suriyavathana and Nandhini, 2010). According to Rammali *et al.* (2022), strains of *Streptomyces* species showed high antimicrobial and antioxidant (ABTS 35.8% and DPPH 25.6%). Furthermore, a study demonstrated a significant ($P < 0.0001$) positive correlation between total phenolic, flavonoid contents and antioxidant capacity, paving the way for characterization of *Streptomyces* sp (Rammali *et al.*, 2022).

Actinomycetes as enzyme sources

Microbial enzymes are enzymes obtained from microorganisms or microbial sources. They are known to be superior enzymes particularly for applications in industries on large scales (Nigam, 2013). Though the enzymes were discovered from microorganisms in the 20th century, studies on their isolation, characterization of properties, production on bench-scale to pilot-scale and their application in bioeconomy have continuously progressed, and the knowledge has regularly been updated (Nigam, 2013). From then many enzymes from microbial sources are already being used in various commercial processes. Selected microorganisms for this use include bacteria, fungi and yeasts, which have been globally studied for the bio-synthesis of economically viable preparations of various enzymes for commercial applications. Amylases, cellulases, chitinases, lipases, pectinases, proteases, and xylanases are secondary metabolites of actinomycetes originated from various environments are good producers of enzymes with enormous economic value. These important biomolecules are useful in food, detergent, medicine, pulp and paper, and textile industries. Most of the commercially applicable enzymes are produced mainly by *Bacillus*, *Pseudomonas*, *Streptomyces* (bacteria), *Aspergillus*, *Trichoderma*, and *Penicillium* (fungi) (Adeoyo, 2020; El-Gendi *et al.*, 2022).

They play major roles in the cycling of organic matter; inhibit the growth of several plant pathogens in the rhizosphere and decompose complex mixtures of polymer in dead plant, animal a fungal material results in production of many extracellular enzymes which are conducive to crop production. The major contribution in biological buffering of soils, biological control of soil environment by nitrogen fixation and degradation of high molecular weight compounds like hydrocarbon in the polluted soils are remarkable characteristics of actinomycetes. Besides this, they are known to improve the availability of nutrients, minerals, enhance the production of metabolites and promote plant growth regulators.

Moreover, actinobacteria do not contaminate the environment instead they help sustainably in improving soil health by formation and stabilization of compost pile formation of stable humus and combine with other soil microorganisms in breaking down the tough plant residues such as cellulose and animal residues to maintain the biotic equilibrium of soil by cooperating with nutrient cycling. Actinomycetes have been shown to be useful in bioremediation, which is the process of using microorganisms to clean up contaminated environment. Examples of bioremediation usefulness of actinomycetes is the biodegradation of hydrocarbon; actinomycetes have been shown to biodegrade a wide range of hydrocarbon including polycyclic aromatic hydrocarbons (PAHs), which are commonly found in soil and water contaminated by petroleum products.

Species such as *Streptomyces griseus* and *Norcardia asteriodes* can effectively degrade PAHs in contaminated environments (Pandey and Chauhan, 2018). Studies have shown that actinomycetes such as *Streptomyces* spp, and *Nocardia* spp, can effectively degrade chlopyrifors in contaminated soil and biodegradation of dyes; Actinomycetes have been shown to degrade a variety of dyes commonly used in textile industry, such as azo dyes. Actinomycetes such as *Streptomyces* spp can effectively degrade azo dyes in contaminated water (Ramakrishna *et al.*, 2018).

2. CONCLUSION

It is clear that majority of industries are looking for microbial strains that can endure different environmental conditions. For enhanced production, actinomycetes species offer a novel paradigm for producing these biomolecules. Since they can produce or secrete a wide range of metabolic substances that are safe for both people and the environment, actinomycetes are extremely valuable. Many genera of actinomycetes have not been modified or investigated for their biotechnological potential. Therefore, when searching for bioactive substances with high economic values for industrial use, this review suggests taking into account these types of organisms.

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