



Thermophilic Actinobacteria from Saudi Arabian Hot Springs: Diversity, Bioactivity, and Bioprospecting Potential

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DOI: 10.31080/ASMI.2026.09.1570

Received: November 27, 2025

Published: December 30, 2025

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Abstract

The growing demand for new bioactive compounds in medicine, industry, and agriculture, coupled with the escalating issue of antimicrobial resistance, has intensified the search for extremophilic bacteria. Saudi Arabia hot springs represent underexplored environments where thermophilic Actinobacteria, adapted to high temperatures and wide range of pH levels, demonstrated remarkable metabolic capabilities and diverse biosynthetic gene clusters. This mini review compiles research (up to 2025) on the diversity, isolation, and biotechnological potential of thermophilic Actinobacteria derived from Saudi Arabian hot springs, including those in Jizan, Al-Lith, Aseer, and Tabuk. Whereas thermophilic *Streptomyces* produce potent antibacterial agents and antifungal agents' metabolites, while actinomycetes isolates produce thermostable enzymes (keratinase, gelatinase, chitinase, and lipase) relevant to textiles, food, and industrial applications. Endophytic actinobacteria obtained from plants growing near hot spring promote plant growth via phytohormones and nutrient mobilization, highlighting their agricultural potential in arid regions. However, previous studies have focused on *Bacillus*, leaving thermophilic Actinobacteria underexplored.

Keywords: Actinobacteria; *Streptomyces*; Bioactive Compound; Saudi Arabia; Hot Springs

Introduction

The growing demand for new bioactive compounds that can be used in industry, medicine, and agriculture has made searching for new microbial sources of antimicrobials agents, anticancer agents, enzymes, and biofertilizers even more urgent. Moreover, the growing resistance of bacteria to antibiotics makes it even more important to find new antibacterial agent therapies [1]. As a result, researchers are exploring extreme habitat such as deep-sea vents, polar regions, and particularly hot springs where extremophilic microorganisms adapted to harsh conditions, exhibit remarkable metabolic capabilities and produce a diver of bioactive compounds [1].

Hot springs typically originate from magma that geothermally heats rainwater or subterranean water near active volcanoes. They are characterized by little salinity and a wide range of pH levels.

These hot springs are suitable habitat for thermophiles that can withstand high temperatures, salinity, acid/alkali stress, and radiation [2,3]. Genomic and physiological studies indicated that numerous hot-spring microorganisms maintain structural stability and enzymatic activity under harsh thermal conditions, highlighting their significant biotechnological potential [2,4]. In this context, thermophilic Actinobacteria have emerged as promising, interesting sources of bioactive molecules with multifaceted applications [4]. Most of the research done so far on Saudi Arabia's geothermal springs has been on *Bacillus* spp [5-8].

This mini review provides information about thermophilic Actinobacteria from Saudi hot springs, focusing on their diversity, isolation, and practical applications, to underscore their potential for bioprospecting and sustainable biotechnology.

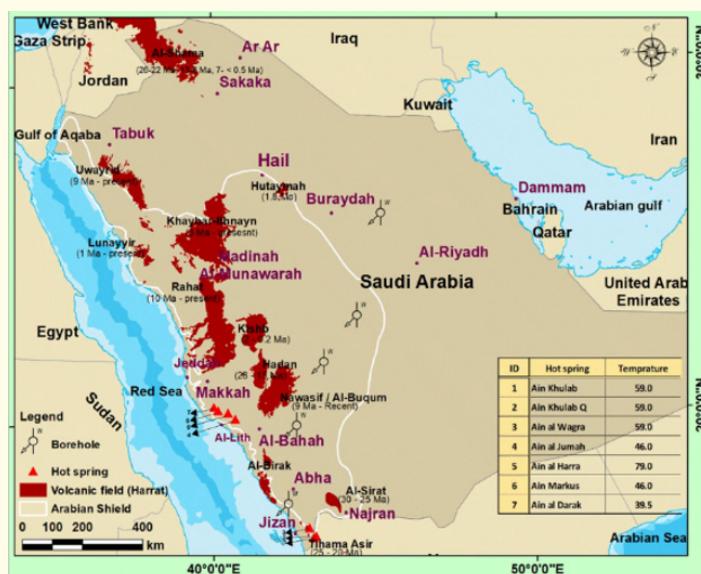


Figure 1: Distribution of hot springs sites in Saudi Arabia, indicated by filled red triangles [9].

Methodology

Literature search strategy

This mini review was performed across Numerous scientific databases, including Scopus, Web of Science, PubMed, ScienceDirect, and Google Scholar, to find relevant publication on thermophilic actinobacteria of hot springs in Saudi Arabia.

The search Expressions that used included:

- Thermophilic Actinobacteria of hot springs in Saudi Arabia
- Actinobacteria of hot springs in Saudi Arabia
- Antimicrobial properties of thermophilic Actinobacteria from hot springs in Saudi Arabia

The literature search covered studies published up to the year 2025 to ensure the inclusion of the most recent and relevant findings.

Actinobacteria: Biological properties and bioactive secondary metabolites

Distribution

Actinobacteria are microorganisms that belong to Bacteria and found in a variety of habitats, where they are prevalent in terrestrial and aquatic habitats and occupy the microbiomes of higher eukaryotes, associated with hosts such as tropical ants, mammals particularly skin and lungs [10,11], and in plants as endophytes, a specialized group of microorganisms that inhabit various internal cells, stems, leaves, and roots without causing illness or damage to the host plant [12]. Furthermore, the actinobacteria also inhabit

freshwater and marine ecosystems, as well as several extreme environments, including hot springs, salt lakes, caves, deep-sea habitats, Antarctica, and desert soils, demonstrating their resilience to severe conditions [3,13].

Their ecological diversity spans a wide range of lifestyles and habitats, including free-living taxa in soil and water (e.g., *Streptomyces*, *Micromonospora*, *Rhodococcus*, *Salinispora*), plant symbionts (e.g., *Frankia* spp.), plant and animal pathogens (e.g., *Corynebacterium*, *Mycobacterium*, *Nocardia*), and gastrointestinal commensals (e.g., *Bifidobacterium* spp.) [14]. This broad distribution shows how organisms can handle drastic changes in pH, temperature, salinity, radiation, and lack of moisture or nutrients [15–17]. Notably, *Streptomyces* species form a large fraction of soil Actinobacteria communities, which historically have been prolific producers of bioactive secondary metabolites [18].

Thermophilic actinobacteria remain underexplored due to challenges associated with their difficulties in isolation and maintenance in pure culture. To facilitate isolation of thermophilic actinobacteria, hot spring samples need more attention and proficiency, i.e., Several chemical and physical pretreatments. Various culture media can be used for isolation, i.e., International Streptomyces Project Media (ISP) [19–21].

Biological characteristics

Actinobacteria are Gram-positive bacteria characterized by their high genomic G+C content ranging from 50% to over 70% and large genomes often around 1–12 Mb [13,22–24]. Their morphological

diversity is diverse coccoid, rod-coccoid, fragmenting hyphae, or branched mycelia form (e.g., *Micrococcus*, *Arthrobacter*, *Nocardia*, and *Streptomyces*) [16]. Actinobacteria inhabit both typical and extreme environments, tolerating a wide range of pH, salinity, acidity, temperature, radiation, moisture, and nutrient limitation. They include aerobic and anaerobic, motile and non-motile forms, as well as spore-forming or non-spore-forming species, where the reproduction occurs through vegetative fragmentation or asexual spores/conidia [15,16]. While many Actinobacteria species are harmless, some species are pathogenic and can cause diseases to humans, animals, and plants [25].

Actinobacteria Bioactive secondary metabolites and their roles

Actinobacteria produces a diverse of bioactive compounds ranging in environmental, agricultural, industrial, pharmaceutical, and clinical fields (Figure 2). The biosynthesis of these metabolites is facilitated by numerous biosynthetic gene clusters (BGCs), that encode the requisite enzymes and regulatory proteins responsible for their production [15,23,25–28]. In agriculture, Actinobacteria contribute to plant health by inhibiting pathogens through antimicrobial agents and insecticidal activities, enhancing plant growth via nitrogen fixation, phosphate solubilization, and phytohormone

Applications & Products of Actinobacteria

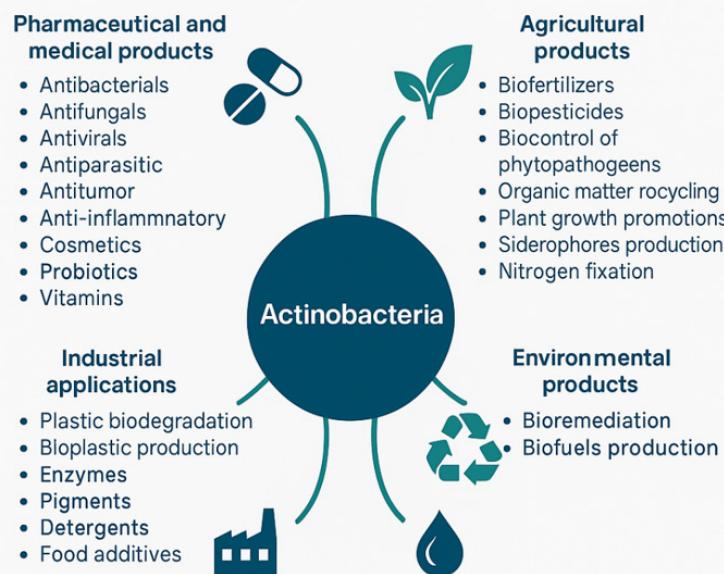


Figure 2: Actinobacteria applications and products (generated by Ai chatgpt.com).

production, and enhancing iron availability and root development. These various functions collectively reduce dependence on synthetic fertilizers [16,29].

Many study worldwide done on the microbial diversity of the hot spring were reported presence of the thermophilic actinobacteria as in study done by Kumar and Sharma [30], examined water samples collected from two hot springs sites, Ringigad and Saldhar in the Garhwal Himalaya, India, their study revealed several actinobacterial species, *Streptomyces albus*, *Streptomyces canescens*, *Thermoactinomyces candidus*, and *Thermoactinomyces thalopo-*

philum were detected in Ringigad, while *Streptomyces albus*, *Thermoactinomyces candidus*, and *Thermoactinomyces thalopophilum* were found and identified in Saldhar. Similarly, Sadeepa., et al. [31] examined microbial diversity of Wahava hot springs, Sri Lanka, Were Actinobacteria identified among the microbial population. In study from Ranong province, in Thailand done by Pansomsuay., et al. [32], analyzed hot springs' sediment and identified strain HSS6-12T, where the taxonomic characterization showed that strain was closely related to the genus *Micromonospora*, where the author he proposed the name *Micromonospora thermarum* for this strain.

Several studies on thermophilic actinobacteria from hot spring demonstrated their ability of these isolate to produce bioactive compound and industrially relevant enzymatic, Sari., *et al.* [33], isolated a strain SL2-2-R-9 obtained from soil sample taken from Cisolok geysers, Sukabumi, West Java in Indonesia, basied on 16S rRNA gene analysis this isolate was identified as *Streptomyces celullosae*, it exhibited antibacterial activity against five gram-positive bacterial strains grown on different growth media, whereas no activity reported against gram-negative bacteria.

Moreover, Kumari., *et al.* [26] investigated sediment samples of Rajgir Hot Springs, Nalanda, Bihar, India. They identified *Actinomycetales bacterium* it is well known to produce various metabolites identified through GC-MS analysis. After ADME/T screening of all metabolites, Diethyl Phthalate (DEP) emerges as the most notable among those generated by the *Actinomycetales*. The isolate showed *in vitro* antibacterial activity against *Listeria monocytogenes* MTCC1143, with a 10-mm zone of inhibition, compared with a 15 mm inhibition zone produced by the reference Ampicillin disc.

Rafiee., *et al.* [1], collected samples from Mahallat Hot Spring in Iran, including water, sediments, sludge, and soils from the surrounding area. Out of the 30 isolates examined, three strains exhibited significant antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Among these, strain Kh3 was identified as *Saccharomonospora azurea*.

Furthermore, Hussein., *et al.* [34] reported three Isolated strains (M1-1, M2-2, M3-2) where obtained from the sediment of Ma'in thermal springs in Jordan. Based on analysis of 16s rRNA , M1-1 found to be related to *Nocardiopsis* sp identity 90% percentage, whereas M2-2 and M3-2 were found to be closely related to *Streptomyces* sp. (97%) and *Nocardioides luteus* (99%), respectively. All three isolates demonstrated clear antimicrobial activity. Isolate M1-1 inhibited the growth of *P. aeruginosa* ATCC 2785, producing a 9 mm zone of inhibition. Strain M2-2 was active against *S. aureus* ATCC 29213 (12 mm), *B. cereus* ATCC 11778 (11 mm), and *E. coli* ATCC 25922 (9 mm). Strain M3-2 showed activity against *S. aureus* ATCC 29213 with a 14 mm inhibition zone and against *B. cereus* ATCC 11778 with a 9 mm inhibition zone.

Enzyme-producing thermophilic actinobacteria have also been frequently reported from hot springs. Cherifa., *et al.* [35], collected water samples taken from the hot springs of Tleghma, located in eastern Algeria. Where the temperature and pH at the sampling site were 58°C and 7.16, respectively. Molecular identification based on 16S RNA gene sequencing revealed that all isolates belong to the *Streptomyces* genus. Among these, nine isolates produced a thermostable α -amylase activity at 55°C.

Furthermore, Fitri., *et al.* [36], isolated Four *Streptomyces* strain from sample collected at Ie Seu'um hot spring, Aceh Besar, Indonesia; these isolates were capable of producing protease, with the highest Proteolytic Index value was 3.8. Chaudhary and Prabhu (2016), isolated thermophilic Actinomycetes From hot spring sample collected at hot springs located in Vajreshwari near Mumbai, Maharashtra, India. These isolates were screened for cellulase and amylase production, and five thermophilic actinomycetes showed a strong capacity to produce lot amounts of thermostable cellulase and amylase, underscoring their potential for industrial applications.

Panosyan [37], conducted a study on sediments obtained from terrestrial geothermal springs in the Akhurik and Tatev regions of Armenia, isolating two *Thermoactinomycetes* strains, identified as AkhA-12 and Tatev 35a. Based on 16S rRNA gene sequence analysis showed both strains belonged to the genus *Thermoactinomycetes*, were they capable of growth at temperatures ranging from 35°C to 60°C, with an optimal pH range of 7.0–7.4, although they could tolerate pH levels between 5.0 and 8.0. Also, they showed salt tolerance, growing at NaCl concentrations of 0–8%, with optimal growth observed at 2–3% NaCl. Notably, the two strains were able to synthesize extracellular hydrolases, including amylase, lipase, and protease.

Thermophilic actinobacteria and its adaptive

Thermophilic and thermotolerant Actinbacteria are present in many genera, such as *Thermopolyspora*, *Thermomonospora*, *Thermotunica*, *Thermocatellispora*, *Thermobispora*, *Acidothermus*, *Acidimicrobium*, and *Thermoleophilum* include only thermophilic species, whilst other genera have both thermophilic and mesophilic species. All these genera are classified into four classes, namely *Actinobacteria*, *Acidimicrobiia*, *Rubrobacteria*, and *Thermoleophilia* of the phylum *Actinobacteria* [16].

Microorgnisms have heat shock proteins to avert protein aggregation at high temperatures, these protiens called chaperones. These chaperones are necessary for preserving protein homeostasis by unfolding denatured proteins that have been compromised by heat [3]. These adaptations make thermophiles significant reservoirs of biodiversity, molecular phylogeny, and secondary metabolite production [38].

Extremophilic actinobacteria exhibit various adaptive strategies, including antibiosis, metabolic mode switching (i.e., autotrophy, heterotrophy, and saprobism), and the synthesis of specific enzymes to withstand adverse environmental conditions (elevated temperature, alkalinity, and salinity). Thermotolerance is attribut-

ed to significant electrostatic and hydrophobic interactions, as well as disulfide bonds, within the proteins of thermophiles. Additionally, they possess certain proteins known as chaperones which aid in refolding the partially denatured proteins [16].

Ecological diversity and biotechnological potential of thermophilic Actinobacteria in Saudi Arabia: insights from hot springs

Hot springs: geochemical setting and microbiological environment

Geothermally heated rainwater or groundwater, usually heated by magma near zones of active volcanism, rises to the surface to form hot springs. These waters typically have low salinity (less than 0.5%) and a broad pH range (0.5–9) [3]. Such conditions provide suitable conditions for thermophilic and hyperthermophilic microorganisms that can survive extreme temperatures, salinity, pH (ranging from acidic to alkaline), and radiation. Thermophilic microbes generally flourish at temperatures between 45 and 80 °C (commonly referred to as 50–80 °C), whereas hyperthermophiles thrive at temperatures above 80 °C [2,38,39].

Saudi Arabian Hot Springs: Variety and Potential

There are about ten known hot springs in Saudi Arabia, mostly located in the regions such as Jizan, Al-Lith, Aseer, and Tabuk. These springs exhibit distinct thermal and chemical features, creating diverse habitats suitable for various extremophilic microorganisms [6,7,38,40]. Community analyses utilizing 16S rDNA sequencing from hot springs in Jizan, Al-Lith, and Tabuk have identified revealed a diverse array of bacterial phyla, including Acidobacteriota, Actinobacteriota, Desulfobacterota, Firmicutes, Halanaerobiaeota, Nitrospirota, and Thermotogota. Among these, Actinobacteria are particularly abundant in Al-Lith soil [40]. These geothermal habitats represent excellent sources for bioprospecting of thermophilic Actinobacteria, which may make thermostable enzymes and bioactive secondary metabolites that have a significant biotechnological and pharmaceutical potential [4,15,28,40,41].

Significant results from Saudi hot springs

A study done on the microbial diversity of Saudi Arabian hot springs conducted by AlSaade., *et al.* [40] were soil samples collected from hot springs located in Jizan, Al-Lith, and Tabuk. Where based on 16S rDNA sequence analysis, the study demonstrated a diverse community of microorganisms thriving in these extreme environments. The study confirmed the presence of *Acidobacteriota*, *Actinobacteriota*, *Desulfobacterota*, *Firmicutes*, *Halanaerobiotae*, *Nitrospirota*, and *Thermotogota*. Among these populations was Actinobacteria the most abundant bacterial population in Al-Lith soils, highlighting the adaptability and ecological importance of this group in thermophilic habitat.

A thermophilic *Streptomyces* strain, designated *Streptomyces* sp. Al-Dhabi 1 was isolated from the Tharban hot spring in the Aseer region of Saudi Arabia. The crude extract of this isolate exhibited notable antimicrobial activity against several pathogenic microorganisms. The minimum inhibitory concentration (MIC) results indicated strong antibacterial activity, with the most potent effects observed against *Streptococcus agalactiae* (<0.039 mg/mL) and *Klebsiella pneumoniae* (0.125 mg/mL). In addition, the extract demonstrated antifungal activity against *Cryptococcus neoformans* (0.078 mg/mL), *Candida albicans* (0.156 mg/mL), *Aspergillus niger* (0.625 mg/mL), and *Trichophyton mentagrophytes* (0.156 mg/mL) [42].

Al-Dhabi., *et al.* [41], conducted a study employ gas chromatography mass spectrometry (GC-MS) to analyze the ethyl acetate extract of *Streptomyces* strain Al-Dhabi 2, which isolated from sediment samples obtained from Tharban hot spring in the southwestern Al-Majardah region of Aseer, Saudi Arabia. The analysis showed that were several bioactive secondary metabolites, with the major compounds identified as benzeneacetic acid (7.81%) and acetic acid, methoxy-, 2-phenylethyl ester (6.01%). This extract showed notable antimicrobial activity, producing inhibition zones ranging from 14 to 25 mm at a concentration of 5 mg/well against the tested pathogenic microorganisms. The minimum inhibitory concentration (MIC) tested showed that the extract had a value of <39 µg/mL against *Bacillus cereus* and *Enterococcus faecalis*, and 78 µg/mL against *Streptococcus agalactiae*. For fungal pathogens, the extract showed MIC values of 156 µg/mL against *Cryptococcus neoformans* and *Trichophyton mentagrophytes*, and 312 µg/mL against *Candida albicans* and *Aspergillus niger*. These findings result indicate that *Streptomyces* strain Al-Dhabi 2 produces potent bioactive metabolites with both antibacterial agents and antifungal agents properties, highlighting its potential as a source of novel thermostable antimicrobial agents.

Furthermore, Bahamdain., *et al.* [43] studded the Oyun Al-Haar hot spring that located in Al-Lith region, which is known to be a rich source of thermophilic microorganisms where eleven bacterial strains were isolated from this spring, among these was nine isolates identified as Actinomycetes. These isolates were characterized and screened for enzyme production, it showed positive results for the synthesis of keratinase, gelatinase, chitinase, and lipase. These enzymes play important role in different industrial applications, especially in the textile and food industries. The finding of thermophilic microorganisms in the Al-Lith hot spring confirms its important potential for biotechnological research.

In addition, Ashkan., *et al.* [44] examined endophytic bacteria that associated with *Heliotropium pterocarpum* plant that growing near the Gomyqah hot spring in Al-Lith region in Saudi Arabia to assess their plant growth promoting (PGP) potential. Among the isolated strains, *Kocuria sediminis* exhibited exceptional enzymatic activity, producing a high level of protease and moderate pectinase activity. This strain also produces a respectable amount of gibberellic acid (GA₃; 0.144 mg/L), which shows that could be good indicator shows the bacterium produce plant-growth promoting through the combined effects of hormone production and extracellular enzyme secretion.

These findings collectively indicate that Saudi Arabian hot springs have variety of thermophilic microorganisms with strong biotechnological potential, producing antimicrobials agents and thermostable enzymes useful in medical, industrial, and agricultural applications. These ecosystems are attractive sources for new bioactive compounds amid rising antibiotic resistance [1,3,41,42,45,46].

Conclusion

In Saudi Arabia, geothermal hot springs offer distinctive environments for thermophilic microorganisms, notably Actinobacteria, which are particularly importance for biotechnological applications, Surveys conducted in places including Jizan, Al-Lith, Aseer, and Tabuk consistently reveal a diversity of microbial communities, among it was Actinobacteriota are the most prevalent. Case studies from these locations have demonstrated that thermophilic *Streptomyces* species produce potent antibacterial agents and anti-fungal agents' metabolites, while actinomycete isolates synthesize thermostable enzymes such as keratinase, gelatinase, chitinase, and lipase. Endophytic isolates from hot spring plants also promote plant growth through phosphate solubilization, siderophore production, and phytohormone synthesis, supporting agriculture in arid areas.

With increasing global challenge of antimicrobial resistance and the increasing need for thermostable industrial enzymes, Saudi Arabian hot springs emerge as promising sites for bioprospecting. While most studies have focused on *Bacillus* species, mounting evidence indicates that thermophilic Actinobacteria possess abundant biosynthetic gene clusters and heat-stable metabolic activities, making them strong candidates for the development of novel pharmaceuticals, enzymes, and biofertilizers.

It is still needed to do more evaluations on antibacterial, anti-cancer, and antioxidant bioassays, as well as on enzyme function. Also, it's still important to show how useful they may be for making new medications, industrial enzymes, and natural agricultural products.

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