IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 14, Issue 6 Ser. III (Nov –Dec 2019), PP 45-54
www.Iosrjournals.Org

Unexplored Extreme Habitats as Sources of Novel and Rare Actinomycetes with Enzyme and Antimicrobial Activities

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Abstract: *Actinomycetes are filamentous or non-filamentous bacteriapossessing high G+C content (>55 mol%) in their nucleic acid. The metabolic diversity of actinomycetes is mainly because of large genome having many transcription factors which enable them to direct gene expression according to precise requirements.Recent genome sequence information suggests that this Streptomyces source of novel compounds is still not yet exhausted.Actinomycetes are ubiquitous organisms with wide physiological and morphological diversity and have been isolated from all kinds of terrestrial and aqueous habitats where they can exist as pathogens or in symbiotic associations with plants and insects or as endophytes. Rare actinomycetes are widely distributed in terrestrial and aquatic ecosystems.Although soil remains their major habitat, rare actinobacteria have also been isolated from sediments, water, plants, stones and animals andthey exist at different environmental settings such as volcanic areas, caves and marine environments. Actinomycetes are producers of secondary metabolites which can be used as medicine. However, there is still an urgent need for discovering novel secondary metabolites to combat the problem of a rising number of resistant pathogenic bacteria and tumor cell linesand increasing need of more efficient enzymes in pharmaceutical industries*.

Key words: *Natural products, Actinobacteria, Streptomyces, secondary metabolites, resistant, rare*

Date of Submission: 17-12-2019 Date of Acceptance: 31-12-2019

I. Introduction

Natural products (NP) play an important role in several sectors of our society because they are valuable for industrial, biotechnological and pharmacological uses in addition to treat human diseases, such as cancer and bacterial infections (Girão *et al*. 2019). Actinomycetes are the most economically and biotechnologically useful prokaryotes and hold a prominent position due to their diversity and proven ability to produce novel bioactive compounds (Subramani and Aalbersberg 2013). In fact, bioactivities reported from actinobacterial NP include antibacterial, antifungal, antitumor, anticancer, anti- inflammatory, antiviral, cytotoxic, and immunosuppressive activities (Girão *et al*. 2019). However, until now, only less than 1% of the actinomycetes have been identified, investigated and documented (Subramani and Sipkema 2019). Isolation and exploitation of actinomycetes for novel compounds from conventional environments have led to rediscovery of known compounds (Jose and Jebakumar 2014). Presently, their share among all known microbial products is only 30~35%, in contrast with the 75~80% of their share during the 1960s to 1980s (Tiwari and Gupta 2012).

Over the last 20 years, there has been a 75% decline in the number of newly approved antibiotics (Ali *et al*. 2018). Due to the decline in the number of new chemical scaffolds and rediscovery of known molecules, the innovation in antibiotic development has slowed down (Subramani and Sipkema 2019). For example, only three new antibacterial classes have been licensed since 1970 (mupirocin in 1985, linezolid in 2000 and daptomycin in 2003)(Ali *et al*. 2018). It is important to speculate on the reasons for the high rate of rediscovery of antimicrobial compounds in previous screening programmes. According to Stach (2010), the reasons are likely to include bias in the screening programmes and limitations in analytical technology, but more importantly in the organisms being screened themselves(Subramani and Aalbersberg 2013).

The chances of isolating undiscovered strains from the terrestrial habitats have diminished so that the search for novel products has switched to rare genera of actinomycetes from normal habitats or to discovery of strains/species found in unusual habitats. The logic behind these approaches is that such strains may be producers of novel bioactive compounds(Khanna *et al*. 2011).The unexplored and underexplored environments are promising sources of rare actinomycetes that are believed to be rich sources of interestingly new compounds (Subramani and Sipkema 2019).In addition, many streptomycetes, although isolated from different environments, evidently produce the same known compounds, probably due to the frequent genetic exchange between them (Subramani and Aalbersberg 2013).

Whole-genome sequencing of several streptomycetes revealed that each member can produce on average 20–30 bioactive small molecules, but only a small fraction of these molecules has ever been detected under various culture conditions(Subramani and Aalbersberg 2013).Consequently, over the past decade, researchers have been attempting several methods such as cloning and heterogonous expression of biosynthetic gene clusters, interfering with regulatory pathways, varying culture conditions, co-culturing two or more organisms together, the adaptive evolution and other strategies to stimulate the production of new compounds(Subramani and Aalbersberg 2013).Although, a single isolate may have the genetic potential to synthesize more than one secondary metabolite, the probability of discovering a novel compound can be far greater if unique isolates are screened simultaneously (Tiwari and Gupta 2012).

II. Actinomycetes from unusual habitats as a source of new compounds

Rare-actinobacteria are commonly categorized as strains other than *Streptomyces* (Berdy 2005) or actinobacteria strains with less frequency of isolation under normal parameters (Baltz2006). Non-*Streptomyces* growth normally slower than *Streptomyces* and requires complicated procedure for isolation, preservation and cultivation in some genera (Lazzarini *et al*. 2000). While discovery of rare actinomycetes may result in increased chances of discovering novel chemical structures, the genetics and physiology of these microorganisms are poorly understood (Tiwari and Gupta 2012).

Non-streptomycete actinomycetes comprise approximately 220 genera up to September 2010(Tiwari and Gupta 2012). According to Tiwari and Gupta (2012), non-streptomycete actinomycetes group comprise diverse bioactive secondary metabolite producing members under following genera: *Actinomadura*, *Actinoplanes*, *Amycolatopsis*, *Dactylosporangium*, *Kibdelosporangium*, *Kitasatospora*, *Microbiospora*, *Planomonospora*, *Planobispora*, *Salinispora*, *Streptosporangium* and *Verrucosispora*. The list has further been extended by reports of bioactive compounds from members of other rare genera, *Nonomuraea*, *Actinoalloteichus*, *Pseudonocardia*, *Saccharothrix*, and *Actinosynnema* (Jose and Jebakumar 2013b).

The rare actinomycetes are considered as a promising source for novel bioactive compounds and hydrolytic enzymes (Benhadj *et al*. 2018).It is clear that isolation of antibiotics and biologically active metabolites has steadily been increasing from rare actinomycetes (Subramani and Aalbersberg 2013). Tiwari and Gupta (2012) reviewed bioactive compounds reported from different genera of rare actinomycetes obtained from various natural habitats. They conclude that many of the successful antimicrobial agents currently available in the market are produced by rare actinomycetes, like rifamycins by *Amycolatopsis mediterranei*, erythromycin by *Saccharopolyspora erythraea*, teicoplanin by *Actinoplanes teichomyceticus*, vancomycin by *Amycolatopsis orientalis*, and gentamicin from *Micromonopsora purpurea* (Subramani and Aalbersberg 2013). Rare or unusual actinomycetes produce diverse, unique, unprecedented and occasionally complicated compounds with excellent antibacterial potency and usually low toxicity (Berdy 2005).Identifying new sources of actinomycetes is a significant approach among the contemporary strategies deal with current need for new antibiotics. Hence, it is indispensable to focus on unexplored unique environments which could have evolved differently from that had already been analyzed (Jose and Jebakumar 2013a).

Search for novel enzymes from unusual ecological niches is often more attractive option leading to development of high-throughput screening programs. Enzymes with new physical and physiological characteristics like high productivity, specificity, stability at extreme temperature, pH or other physiological conditions, low cost of production, and tolerance to inhibitors are always most sought after properties from an industrial standpoint.Studies on unique ecological environments could yield molecules that could become future harbingers of green technology (Prakash *et al*. 2013).

III. Diverse habitats of actinomycetes

Actinomycetes are the most widely distributed group of microorganisms in nature which primarily inhabit the soil. Apart from soil, they are found in marine and terrestrial environments(Rana and Salam2014). Although members of this large phylum exist as free-living saprophytes, several of them can live inside tissues or organs as commensal or symbiotic partners of plants, insects, aquatic animals as well as terrestrial animals and human beings (Lacombe-Harvey *et al*. 2018).

3.1 Soils

Actinomycetes are found abundantly in all soils throughout the world such as alkaline soil, desert soil, soils from salt pans to under the snow caps(Agarwal and Mathur 2016). Environmental factors such as soil type, pH, humus content, and the characteristics of the humic acid content of the soil affect their distribution (Tiwari and Gupta 2012).

Actinomycetes were isolated from rhizosphere soil samples collected from different regions of Madhya Pradesh state. Out of 85 actinomycetes, only 5 actinomycetes showed pigment production and based on diffusible pigment production ability one actinomycete ARITM02 was selected.The study confirmed that the natural pigment has very less cytotoxic effect and probably used in food and pharma industries as a natural colorant agent. Compound is more effective against cancer cell lines as compared to normal cell lines.The result indicates that the pigment has antagonistic activity against microorganisms including bacteria, yeast and molds.The pigment also has good antioxidant activity and could be used further as an antioxidant compound (Parmar and Singh 2018). A moderately thermotolerant *Streptomyces atrovirens* subspecies isolated from a soil sample collected on Jeju Island, Korea (strain WJ-2) was an excellent producer of extracellular xylanases (Kim *et al*. 2016).

3.2. Aquatic environments

Microbial communities inhabiting aquatic environments vary according to the physiochemical parameters including temperature, salinity, pH and nutrient loads (El-Gayar *et al*. 2017). Actinomycetes are predominant in river and lake and marine environments, despite some of them being introduced from terrestrial habitats (Subramani and Aalbersberg 2013).

a- Fresh environments

Xu and Jiang (1996) studied actinomycete populations of 12 lakes in the middle plateau of Yunnan (China) and found that *Micromonospora* was the dominant genus (39–89%) in the actinomycetes population in sediments of those lakes. Furthermore, *Streptomyces* was the second most abundant genus. 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 lake sediments.Several workers confirmed the presence of *Micromonospora* in streams, rivers and lake sediments. *Micromonospora* had a role in the turnover of cellulose, chitin and lignin (Chavan *et al*. 2013).

InIndia, 10 actinomycetes were isolated fromtheestuaryand later five were selected for secondary screening and noted significant activity against *Enterobacter aerogenes* and *Proteus mirabilis*. Among the selected *Streptomyces* sp., ES2 showed potent activity against selected microbes and was identified as *Streptomyces* sp. The studied isolates were resistant towards streptomycin (10 μg), ampicillin (50 μg) and ciprofloxacin (5 μg) (Al-Ansari *et al*. 2019).

Overall, the study revealed that the selected aquatic rare actinomycetes recovered from Fetzara Lake presented good candidates to be explored as new sources of bioactive compounds.Interestingly, significant anticandidal and antibacterial activities against both Gram-positive and Gram-negative bacteria were observed. Furthermore, the actinomycetes isolates were able to produce different hydrolytic enzymes with potential industrial and food processing applications such as amylase, cellulase, protease, and lipase (Benhadj *et al*. 2018).

From the present study, it is concluded that the sediments of the shrimp pond are good source materials for the isolation of potential actinomycetes. The present study has revealed that the tentatively identified species, *S. aureofasciculus* isolated from the sediments possesses good α-amylase activity (Poornima *et al*. 2008).

b- Marine environments

The sea has a number of unique marine habitats including sea-grass beds, salt-pans, mangroves, coral reefs, salt marshes, numerous fish species and different microbial communities (Abdelfattah *et al*. 2016). The best marine source of actinomycetes is sediment and also reported from water, sand, rocks, seafood's, marine plants, mangrove sediment and deep sediment(Chavan *et al*. 2013).The culture ability of microorganisms from seawater is considerably lower (0.001–0.10 %) than that from marine sediments (0.25 %) (Subramani and Aalbersberg 2013). Sediment collected from a depth of 4920 meters in the Atlantic Ocean 500 miles from land was found to contain small numbers of thermo actinomycetes (Chavan *et al*. 2013).The highest numbers of natural products were derived from the genera *Nocardiopsis, Micromonospora, Salinispora* and *Pseudonocardia*. Members of the genus *Micromonospora*were revealed to be the richest source of chemically diverse and unique bioactive natural products(Subramani and Sipkema 2019).

Most of the compounds produced by the marine rare actinomycetes present antibacterial, antifungal, antiparasitic, anticancer or antimalarial activities. The solvent extract of isolates from Thoothukudi coastal ecosystem showed significant reduction in the growth of pathogenic organisms, which paves way for commercial exploitation of the isolates to control plant pathogens (Dhevagi *et al*. 2017). Prudhomme *et al*. (2008)reported the potential of secondary metabolites, derived frommarine microorganisms, to inhibit *Plasmodium* growth.

Several hundred species of *Streptomyces* were isolated from sea water, marine sediments including mangroves, marine mollusks and detritus. One of the significant observations was that nearly 70% of *Streptomyces* sp. isolated from marine mollusks was antagonistic, whereas only 20-25% of cultures isolated from sediments showed antagonism towards the test microorganism(Chavan *et al*. 2013). The *Streptomyces* sp. isolated from marine sediment samples showed potent antifungal activity (Karthik *et al*. 2010). Lynamicins A-E are chlorinated bisindole pyrroles isolated from the rare actinomycete *Marinispora* sp. The antimicrobial spectrum of lynamicins was evaluated against a panel of 11 pathogens, which demonstrated that these substances possess broad spectrum activity against both Gram-positive and Gram-negative pathogens.

Significantly, lynamicins were active against drug resistant pathogens such a methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium* (Subramani and Aalbersberg 2013).

Another research leads to discovery of a novel alkaloid, xinghaiamine A, from a marine derived actinomycete *Streptomyces xinghaiensis* NRRL B24674T. Biological assays revealed that xinghaiamine A exhibited broad spectrum antibacterial activities to both Gram-negative persistent hospital pathogens (e.g. *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Escherichia coli*) and Gram-positive ones, which include *Staphylococcus aureus* and *Bacillus subtilis*. In addition, xinghaiamine A also exhibited potent cytotoxic activity to human cancer cell lines of MCF-7 and U-937 with the IC50 of 0.6 and 0.5 mM, respectively(Abraham and Chauhan 2017).

Some marine actinomycetes were isolated from costal soil samples by serial dilution and spread plate method. From them, *Streptomyces paradoxus* VITALK03 exhibits antidiabetic activity by inhibiting α-glucosidase and α-amylase enzymes, and prevents hemoglobin glycosylation. It also exhibits free radical scavenging antioxidant activity. The antidiabetic and antioxidant potential of the isolates can be explored further to develop as an effective antidiabetic agent (Ravi *et al*. 2017).

The sediments collected from various places of Bay of Bengal coast was subjected to actinomycete isolation. The isolate ACT1 was identified as a species of the genus *Streptomyces* sp., based on microscopic, cultural and biochemical characterization. However, the fractionated extract (ethyl acetate, ethanolic, acetone and hexane fraction), especially ethyl acetate fraction, showed varied biological activities such as antimicrobial activity and enzyme production. The actinomycete isolate *Streptomyces* sp. can be a potential candidate for the development of therapeutic agents (Iswarya *et al*. 2018).A salt tolerant alkaliphilic actinomycete *Streptomyces clavuligerus* strain Mit-1 isolated from Mithapur, western coast of India has been reported to produce alkaline protease, and so as the alkaliphilic actinomycetes isolated from marine sediments of the Izmir Gulf, Turkey, strain MA1-1 (Solanki and Kothari 2011). A good member of culturable strains of *Streptomyces*is capable of producing different enzymes viz. L-asparaginase, cellulase, DNase and chitinase (Gobalakrishnan *et al*. 2016).Actinomycetes from marine sources have been reported to decompose agar, alginates, cellulose, chitin, oil and other hydrocarbons. They have been also implicated in the decay of wood submerged in seawater (Chavan *et al*. 2013).

3.3.Mangrove ecosystem

Mangrove sediments are an abundant source of actinomycetes population having versatile producers of various enzymes and antimicrobial molecules (Subramani and Aalbersberg 2013).Novel actinomycetes reported from different mangrove habitats including sediments, mangrove plant rhizosphere soil and mangrove endophytes are classified into 25 genera, 11 families and 8 suborders (Subramani and Sipkema 2019).

A novel aerobic actinomycete, designated HA11110T, was isolated from a mangrove soilsample collected in Haikou, China. 16S rRNA gene sequence similarity showed that strain HA11110T belonged to the genus *Streptomyces*, most closely related to *Streptomyces fenghuangensis* (99.1 %), *Streptomyces nanhaiensis* (98.8 %) and *Streptomyces radiopugnans* R97T (98.8 %). On the basis of phenotypic and genotypic data, strain HA11110T represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces mangrovi* sp. nov. was proposed (Abraham and Chauhan 2017).

A total of 14 new rare actinomycete species belonging to seven different families have been reported in mangrove sediments from the period 2007-2013(Subramani and Aalbersberg 2013). Different genera such as *Brevibacterium, Dermabacter, Kytococcus, Microbacterium, Nesterenkonia,* and *Rothia* were isolated from mangrove sediments in Brazil(Dias*et al*. 2009). In China, a number of rare actinobacteria including *Actinomadura, Isoptericola, Microbispora, Nocardia, Nonomuraea,* and *Rhodococcus* were isolated from mangrove soils and plants (Abraham and Chauhan 2017).

Rare bioactive actinomycetes were isolated from unexplored regions of Sundarbans mangrove ecosystem and possess 93.57 % similarity with *Streptomyces albogriseolus* NRRL B-1305 and possess good antimicrobial and antioxidant activity (Abraham and Chauhan 2017). Raja *et al*. (2010) isolated 17 marine actinobacteria strains from the rhizosphere sediments of mangroves and reported the amylase inhibitor.

3.4. Actinobacterial symbionts of plants and animals

a- Endophytic actinomycetes

Previous studies demonstrated that a variety of *Streptomyces* inhabit a wide range of plants as either symbionts or parasites and play a crucial role in plant development and human health. (Lee*et al*. 2014). For example, Qin *et al*. (2009) reported for the first time the isolation of *Saccharopolyspora*, *Dietzia, Blastococcus, Dactylosporangium, Promicromonospora, Oerskovia, Actinocorallia* and *Jiangella* species from endophytic environments. Several rare actinomycetes have also been isolated from lichen samples collected in Japan and were proposed as new species (Tiwariand Gupta2013).

Endophytic actinomycetes have been explored in the recent years as a potent antibiotic producer. They can be isolated from the disinfected surfaces of plant tissues or that can be extracted from within the plant (Siva *et al*. 2011). For example, new anti-trypanosomal compounds, the spoxazomicins, have been found in the culture broth of a novel endophytic actinomycete (Tiwari and Gupta 2013). *Streptomyces parvulus* Av-R5 associated with root of *Aloe vera* exhibited the highest activity against multidrug-resistant *Staphylococcus aureus*, *Staphylococcus epidermidis*,*Klebsiella pneumoniae* and *Aspergillus niger*(Chandrakar and Gupta2019).Reduced incidence of root infection has been correlated with an increase in number of *Streptomyces* in the rhizosphere which inhibits the pathogen by production of antifungal antibiotics and *Actinoplanes* can act as biological control agents of plant diseases (Chavan *et al*. 2013).

A total of 45 Brazilian actinomycetes previously isolated from plants (endophytics) and soil were prospected for hemicellulases and β-glucosidase production (Robl *et al* 2019).The bacterium *Streptomyces* sp. DPUA1566 isolated from lichens from the Brazilian Amazon was found to produce a lipoprotein biosurfactant. The biosurfactant proved to have effective surface tension reduction capacity and emulsification activity toward hydrocarbons and vegetable oils. Its thermal stability, tolerance to wide ranges of pH and salt concentration and absence of toxicity makes this biosurfactant a promising candidate for applications in biotechnological, environmental, cosmetic, food and pharmaceutical industries (Santos *et al*. 2019).

b- Animal-associated actinomycetes

Insect-associated *Streptomyces* inhibit antimicrobial-resistant pathogens more than soil *Streptomyces*. Genomics and metabolomics reveal their diverse biosynthetic capabilities. Further, cyphomycin, a new molecule active against multidrug resistant fungal pathogens was described (Chevrette *et al*. 2019). Two antifungal compounds were produced from ant associated actinomycetes (Subramani and Aalbersberg 2013). The microhabitat approach was used in a study to explore the bacterial diversity of dissected tissues from venomous cone snails (Tiwari and Gupta 2013). Their results revealed a diverse, novel, and highly culturable cone snail-associated actinomycete community, with some isolates showing promising bioactivity in a neurological assay.

c- Marine symbiotic actinomycetes

Symbiotic microorganisms, especially actinomycetes from marine invertebrates, plants and animals, are now rapidly emerging for drug discovery programmes (Subramani and Aalbersberg 2013).

A total of 17 new rare actinomycete species belonging to 11 different actinomycete families have been reported in plants and animals, respectively, between 2007 and mid-2013. Among them, five novel genera *Labedella, Phycicola, Iamia, Euzebya* and *Koreibacter* were reported from marine algae and animals(Subramani and Aalbersberg 2013). *Laminaria ochroleuca* (marine macroalgae)is a rich source of actinobacteria with promising antimicrobial and anticancer activities and suggests that macroalgae may be a valuable source of actinobacteria and, consequently, of new molecules with biotechnological importance (Girão *et al*. 2019).

Some 14 putatively novel species of actinomycetes were isolated from 11 different species of marine sponges that had been collected from offshore Ras Mohamed (Egypt) and from Rovinj (Croatia) (Tiwari and Gupta 2013). Another study described actinomycetes isolated from the marine sponge *Haliclona* sp. collected in shallow water of the South China Sea using selective media. A novel actinomycete, *Tsukamurella spongiae*, was isolated from a deep-water marine sponge collected off the coast of Curaçao in the Antilles, Netherlands (Tiwari and Gupta 2013).

An antibiotic-producing actinobacterium, was isolated from marine sponge from São Paulo, Brazil and the isolate was classified as the type strain of a novel species of the genus *Williamsia*, for which the name *Williamsia aurantiacus* sp. nov. is proposed (de Menezes *et al*. 2019).The sponge associated actinomycetes, *Nocardiopsis dassonvillei* having 100% activity against multidrug resistant pathogens have been reported (Selvin *et al*. 2009). They have isolated 11 antimicrobial compounds in *N. dassonvillei* MAD08 and also isolated an anticandidal protein of molecular weight 87.12 kDa and it has been reported that this is the first strain that produces both organic solvent and water soluble antimicrobial compounds. Thiochondrillines, analogs of thiocoraline, are potent cytotoxic thiodepsipeptides isolated from the sponge associated *Verrucosispora* sp. (Subramani and Aalbersberg 2013). *Streptomyes spongiicola* HNM0071T is a novel marine sponge-associated actinomycete with potential to produce antitumor agents including staurosporine and echinomycin (Zhou *et al*. 2019). Caerulomycin A- antifungal potential was isolated from marine invertebrate-associated *Actinoalloteichus* sp. using optimized medium and fermentation conditions (Abraham and Chauhan 2017).

3.5. Extreme environments

The extreme habitats are characterized by chemical or physical conditions that differ significantly from those found in environments that support more abundant and varied life forms (Jose and Jebakumar 2014) (Figure 1). Microorganisms, including actinomycetes, adapt and grow in various ecological niches such as low temperatures in glaciers and the deep sea, acidic and alkaline pH in the industrial and mine wastewater effluents, high levels of radiation and extreme desiccation indeserts, high salt concentration in lakes, and high temperatures in hot springs and thermal vents (Mahajan and Balachandran 2017).

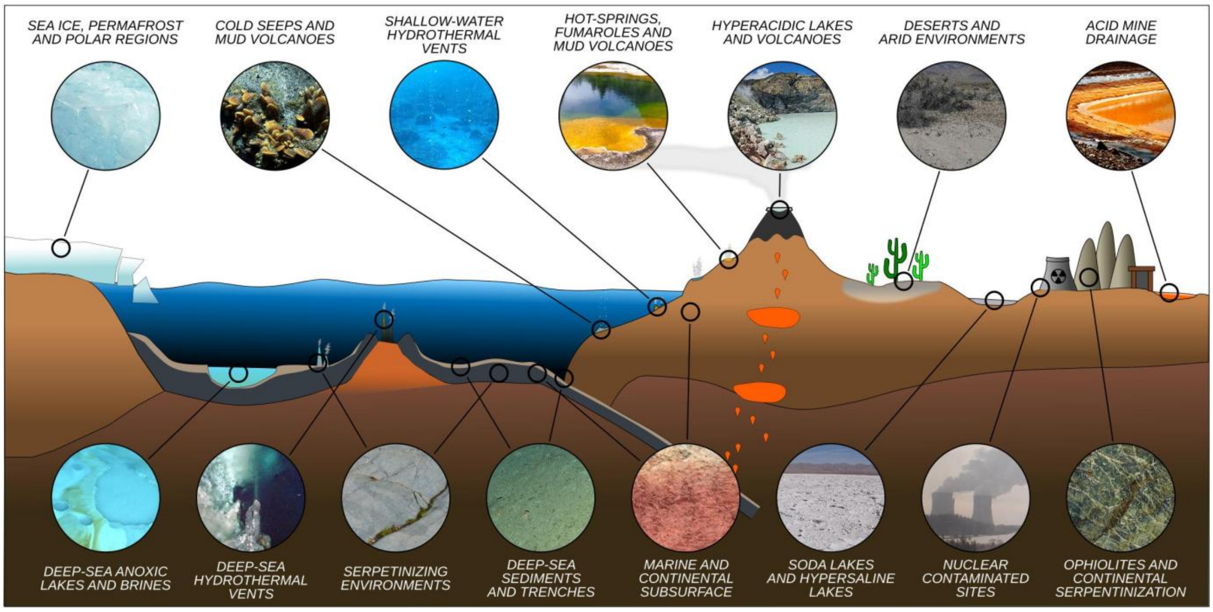

Figure 1. Representative idealized cross section of Earth's crust showing the diversity of extreme environments and their approximate location (Merino *et al*. 2019).

3.5.1 Hypersaline habitats

Halophiles are distributed in hypersaline environments all over the world, mainly in natural hypersaline brines in arid, coastal, and deep-sea locations as well as in artificial salterns. These extremophiles have been found in a variety of microenvironments including salt lakes, salinesoils, cold saline environments, alkaline saline habitats, and salted fish, meat and other foods (Ara *et al*. 2013).

In addition to *Streptomyces*, strains belonging to *Micromonospora, Saccharothrix, Streptosporangium,* and *Cellulomonas* were obtained from the Qinghai-Tibet Plateau (Ding *et al*. 2013), while*Micromonospora, Actinomadura*, and *Nocardiopsis* were reported from soda saline soils of the ephemeral salty lakes in Buryatiya (Lubsanova *et al*. 2014).Solar salterns are unique hypersaline environments, characterized by their high salt concentration and alkaline pH.A total of 14 slow growing actinomycetes were selectively isolated from three composite soil samples of inland solar salterns. They were screened for their antimicrobial activity against a range of microorganisms (Jose and Jebakumar 2013a). Tian *et al*. (2013) reported the isolation and characterization of p-Terphenyls with antifungal, antibacterial, and antioxidant activities from halophilic actinomycete *Nocardiopsis gilva* YIM 90087. Strains of halophilic actinomycetes have the potential of secreting extracellular enzymes (protease, lipase, esterase, galactosidase, amylases, etc.), which work well in alkaline pH range, tolerating high concentrations of organic solvents in their environment (Solanki and Kothari 2011).

3.5.2 Caves

Recently, several new species of actinomycetes have been isolated from caves, including from a gold mine in Korea, the Reed Flute Cave in China, the Grotta Dei Cervi Cave in Italy and a cave occupied by bats in Spain (Subramani and Aalbersberg 2013). At the time of writing, 47 species in 30 genera of actinobacteria were reported from cave and cave related habitats (Rangseekaew and Pathom-aree 2019).

A novel actinobacteriumwas isolated from a soil sample collected from a karst cave in, China. On the basis of phenotypic, genotypic and phylogenetic data, it was a novel species of the genus *Nocardioides* (Zhang *et al*. 2018).The novel rare actinomycete genera *Beutenbergia* and *Terrabacter* have been reported from small stones collected from caves and agricultural fields, respectively (Subramani and Aalbersberg 2013). Actinobacteria often colonize the rock walls of caves. In a study on biogeochemical role of actinobacteria in Altamira Cave (Spain), Actinobacteria-coatedspots on the cave walls was found to uptake carbon dioxide gas which is available in abundance in cave. This gas is used by the bacteria to dissolve rock and subsequently generates crystals of calcium carbonate (Fang *et al*. 2017).

Antagonistic *Streptomyces, Micromonospora, Streptosporangium* and *Dactylosporangium* were isolated from five caves (Cheondong, Kosoo, Nadong, Seonglyu, and Ssangyong) in Korea. They showed activity against at least one of plant pathogenic fungi (*Alternaria solani, Colletotrichum gloeosporioides, Fusarium oxysporum* and *Rhizoctonia solani*). Similarly, members of genera *Streptomyces* and *Janibacter,* isolated from limestone deposit sites in Hundung, Manipur, India were reported to show anticandidal and biocontrol activities against rice fungal pathogens (*Curvularia oryzae, F. oxysporum, Helminthosporum oryzae,* *Pyricularia oryzae* and *R. solani*) as well as antibacterial activity (Rangseekaew and Pathom-aree 2019). *Streptomyces* E9 isolated from Helmcken Falls cave in British Columbia could inhibit the growth of *Paenibacillus* larvae, a causative agent of American foulbrood disease in honeybees (Rangseekaew and Pathom-aree 2019). Cervimycin A, B, C, and D were produced from *Streptomyces tendae* strain HKI 0179, isolated from a rock wall. Xiakemycin A is a novel antibiotic produced by *Streptomyces* sp. CC8-201 from soil in China. Xiakemycin A showed strong inhibitory activitiesagainstGrampositivebacteriaand cytotoxic against numerous cancer cell lines (Rangseekaew and Pathom-aree 2019).

3.5.3 Extreme cold habitats

The extreme environment of low temperature is one of the major abiotic stresses acting as the limiting factor affecting the agricultural productivity. 20% of the Earth's surfaces were covered frozen soils (permafrost), glaciers and ice sheets, and snow cover area (Yadav *et al*. 2019).Bacterial populations in Roopkund Glacier, Himalayan mountain were studied andactinobacteria is the predominant class, followed by β- proteobacteria (Rafiq *et al*. 2017).As these environments considered being the greatest diversity of culturable actinomycetes, studies in the recent past revealed the occurrence of novel *Streptomyces* spp. from the Antarctic ecosystem (Sivalingam *et al*. 2019).Two novel actinomycetes, designated strains ZLN81T and ZLN712T, were isolated from a frozen soil sample which was collected from the Arctic region(Kamjam *et al*. 2019).

Some actinomycetes were isolated from rhizosphere soil from Lachung, Himalaya region and exhibiting antimicrobial activity. Out of the total isolates, 17 (66%) isolates showed antimicrobial activity and all the isolates produce at least one extracellular enzyme(Singh*et al*. 2019).Bacterial diversity of soil samples from Drass, India a coldest place after Siberia, was explored and screened for various hydrolytic enzymes. Phylogenetic analysis revealed 40 different bacteria, grouped into three major phyla, Proteobacteria, Actinobacteria and Firmicutes differentiated into 17 different genera. These isolates were also investigated for production of hydrolases at 4–30˚C. All the isolates secreted one or the other hydrolytic enzyme, i.e. esterase (90%), lipase (80%), protease (32.5%), amylase (20%) and cellulase (17.5%) (Rafiq *et al*. 2017).

3.5.4Thermophilic habitats

Although habitats with elevated temperatures are not as widespread as temperate or cold habitats, a variety of high temperature, natural and man-made habitats exist. These include volcanic and geothermal areas with temperatures often greater than boiling, sun-heated litter and soil or sediments reaching 70°C, and biological self heated environments such as compost, hay, saw dust and coal refuse piles (Agarwal and Mathur 2016).

Some microbiologically diverse and specialized habitats for the isolation of thermophilic actinomycetes are desert soil, hot springs, volcanic eruptions and thermal industrial wastes (Agarwal and Mathur 2016).In recent years, researchers have shown great interest in thermophilic actinomycetes because of their economic potential, either in useful biological processes such as biodegradation, or in the production of antibiotics and enzymes(Agarwal and Mathur 2016). Enzymes from these microorganisms also got special attention from the scientist from all over the world since these enzymes resistant to chemical reagents and extreme pH values in comparison to their mesophilic homologues (Akmar *et al*. 2011).

Thermoactinomycets belong to genus *Thermoactinomyces, Thermomonospora, Microbispora, Saccharopolyspora* and *Streptomyces*. Among these thermophilic actinomycetes, the genus *Thermoactinomyces* has industrial and clinical importance. Some *Thermoactinomyces* strains are known as potent protease producers (Agarwal and Mathur 2016). A number of hydrolytic enzymes such as amylases, xylanases and cellulase from thermotolerant actinobacteria can maintain their enzymatic activity, even at high temperatures (50–65^{o}C) (Mohammadipanah and Wink 2016).

a-Hot Springs

The hot spring sediments are a great source for discovery of new actinomycetes and the bioactive compounds (Thawai2012).During our study on thermophilic actinobacterial resources from hot springs, the strain YIM 78087T was isolated from a sediment sample collected from Hehua hot spring in Yunnan province, southwest China. The experimental data we obtained also indicated that isolate YIM 78087T represents a novel species of the genus *Streptomyces*, named here *Streptomyces calidiresistens* sp. nov (Duan *et al*. 2014).

In this study, we successfully isolated the actinomycetes from the sediments collected from hot spring pond located in Krabi and Trang province, Thailand. These actinomycete strains were identified using the morphological property and 16S rRNA gene sequence analysis. They belonged to the member of genera *Streptomyces, Micromonospora, Microbispora* and *Planosporangium*. The crude ethyl acetate extract from the fermentation broth of the representative strain in each group with exception in the genus *Planosporangium* exhibited the antibacterial activity against Gram-positive bacteria i.e. methicillin resistant *Staphylococcus aureus* (MRSA), *Micrococcus luteus* ATCC 9341 and *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633 (Thawai 2012).

Abussaud *et al*. (2013) investigated the antimicrobial activity in 8 thermophilic *Streptomyces* strains isolated from hot springs; the strains inhibited the growth of *E. coli*, *S. aureus* and *C. albicans*. *Streptomyces* sp Al-Dhabi-1 isolated from hot spring of Saudi Arabia showed good antimicrobial activity against tested microbes in preliminary screening (Al-Dhabi *et al*. 2016). The thermophilic *Streptomyces* strain was isolated from thermal spring in Saudi Arabia and identified based on standard methods via using phenotypic and molecular identification techniques. The phenotypic and molecular characteristics of Al-Dhabi-2 are consistent with those of the genus *Streptomyces*. Al-Dhabi-2 exhibited moderate antibacterial and antifungal activities in the streak method(Al-Dhabi*et al*. 2019).Hot spring sediment and soil samples total of twenty samples from West Anatolia in Turkey were investigated for the occurrence of thermophilic actinomycetes. Strains were grown at 55°C. Sixty-seven thermophilic actinomycetes isolates were classified in *Thermoactinomyces thalpophilus* and *T. sacchari* species. Among these, maximum isolates were found to be extracellular protease producers(Agarwal and Mathur 2016). Actinomyces species from hot water spring which produce remarkable amount of thermostable amylase and cellulase, active at acidic and alkaline pH (Chaudhary and Prabhu 2016).

b-Volcanic crusts

Although very studies showed the presence of actinobacteria in these areas but a decent research on such bacteria could be of great significance (Agarwal and Mathur 2016). The taxonomic position of two actinomycetes strains, LC2T and LC11T, isolated from a filtration substrate made from Japanese volcanic soil, was determined using a polyphasic approach. The strains grew at temperatures from 5 to 45^{0} C. A phylogenetic tree based on 16S rRNA gene sequences showed that the two strains formed a distinct evolutionary lineage within the genus *Amycolatopsis*. The isolates are proposed to represent two novel species(Agarwal and Mathur 2016). Two thermophilic *Rhodococcus* and *Streptosporangium* were isolated from a mud volcano in India (Mohammadipanah and Wink 2016).It is evident that volcanic spring is one of the extreme habitats on Earth and harbours novel microbes as a source of potential drug leads. Importantly, although there have been few notable studies on isolation of natural drugs from volcanic *Streptomyces*, the knowledge of *Streptomyces* population in volcanic habitat is sparse (Sivalingam *et al*. 2019).

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Magda M. Aly. "Unexplored Extreme Habitats as Sources of Novel and Rare Actinomycetes with Enzyme and Antimicrobial Activities." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 14.6 (2019): 45-54