Chapter 2
Review of Literature

What you learn from a life in science is the vastness of our ignorance.

David Eagleman

2.1 Soil an Ecosystem

Soil microorganisms are greatly important in the soil food web and natural equilibrium (Neemisha 2020). Microorganisms in soil are significantly relative to healthy soil and healthy plant because they are a considerable component of soil physical and chemical processes. The soil microbial communities can improve soil structure for plant growth by enhancing soil aggregate stability (Lu et al. 2018). Bacterial polysaccharides and, fungal hypha and metabolic products play important role in binding soil particles together (Costa et al. 2018, Haynes et al. 2020; de Caire et al. 1997; Caesar-TonThat and Cochran 2000). Soil aggregation is necessary for soil quality to improve infiltration rate, water holding capacity

and plant root development. Thus, decreasing microbial biomass and their activities are a result of reducing soil aggregation (Gao et al. 2019; Jain & Saxena 2019). Numerous species of microorganisms including protozoa, bacteria, fungi and nematode are contained in the soil. However, bacteria and fungi seem to be the most important in soil nutrient cycling because they are the first organisms degrading organic materials as their energy source (Rashid et al. 2016; Kladivko 2001). Soil fertility is enhanced by increasing microbial biomass. Soil microbes strongly influence soil biogeochemical cycles because they obviously express their ability in organic matter decomposition, nutrient mineralization and nutrient cycling (Witzgall et al. 2021). Furthermore, some soil microorganisms have the potential to degrade or detoxify chemical pollutants and pesticides in soil (Raffa & Chiampo 2021). However, the structure of the microbial community depends on many factors such as climate, moisture, topography, plant growth, and quantity and quality of substrates (Deltedesco et al. 2020) Additionally, soil chemical (e.g., pH and salinity) and physical condition (e.g., texture and soil-water potential) are also influence soil microbial efficiency (Almendro-Candel 2018). Hence, the soil microbial community can respond to environmental change and it has been used for monitoring the impact of agricultural practices and ecological stresses on soil health (Alori et al. 2020). The reservoir of soil microorganisms in the pore structure within and between soil particles. The community of soil microorganisms can use as a useful indicator of soil quality and ecological stresses because of their adaptation.

2.2 The Actinobacteria

Actinobacteria, previously known as ‘Actinomycete’ or ray fungi is originated from two Greek words ‘atkis’ (ray) and ‘mykes’ (fungi). It forms an important constituent of the microbial biome and comes next to proteobacteria in terms of number and distribution. Actinobacteria are mostly aerobic, gram-positive to gram variable with high G+C content and occupy diverse microbial niche (Amin et al. 2020). The phylum also includes a few

Gram-negative species such as *Thermoleophilum* sp. (Zarilla & Perry 1986), *Gardenerella vaginalis* (Gardner & Dukes 1955), *Saccharomonospora viridis* P101T (Shin et al. 2017), *Ferrimicrobium acidiphilum,* and *Ferrithrix thermotolerans* (Johnson et al. 2009). They share some characteristics with fungi, such as colony morphology, mycelial growth and musty smell, on the other hand, peptidoglycan cell wall structure is common with bacteria. On this basis, it was named as 'Actinobacteria' by C. O. Harz and it was considered as the missing link between bacteria and fungi. Though the first Actinobacteria was discovered by Ferdinand and Cohen from human lachrymal ducts in the year 1875 it came to the limelight only after the discovery of the antibiotic, actinomycin by Dr. Selman Waksman A phylogenetic study based on 16S rRNA classifies Actinobacteria into six classes i.e., Acidimicrobia, Coriobacteria, Nitriliruptoria, Rubrobacteria, Thermoleophilia and Actinobacteria (Sen et al. 2014). So they were considered transitional forms between fungi and bacteria. Indeed, like filamentous fungi, many *Actinobacteria* produce a mycelium, which is nonseptate and slenderer and many of these mycelial Actinobacteria reproduce by sporulation (Chater & Chandra 2006). However, the comparison to fungi is only superficial: like all bacteria, Actinobacteria cells are thin with a chromosome that is organized in a prokaryotic nucleoid and a peptidoglycan cell wall; furthermore, the cells are susceptible to antibacterial agents (Smith 2005)). Physiologically and ecologically, most Actinobacteria *a*re aerobic, but there are exceptions (*Actinomycosis meyeri* and *A. israelii*). Further, they can be heterotrophic or chemoautotrophic, but most are chemoheterotrophic and able to use a wide variety of nutritional sources, including various complex polysaccharides (Shivlata & Satyanarayana 2015).The genome size of Actinobacteria ranges from 0.93 Mb (*Tropheryma whipplei*; Bentley et al. 2003) to 12.7 Mb (*Streptomyces rapamycinicus*; Baranasic et al. 2013), that exists either as a circular or linear form. Actinobacteria mostly inhabit soil, freshwater, and marine habitats playing a pivotal role in the

disintegration of organic materials, such as cellulose and chitin, thereby playing a vital part in biogeochemical cycles, refilling the supply of nutrients in the soil, and helps in humus formation.(Elbendary et al. 2018) They have been considered as one of the significant groups of microorganisms as they represent a broad range of valuable and prominent sources of pharmaceutically active metabolites. These metabolites are analyzed by GC-MS technique and are utilized as antimicobial and anticancerous agents. (Ajilogba et al. 2019)

2.3 Diversity and importance in various ecosystems

Actinobacteria are widely distributed in various biotopes such as soil, water, permafrost, mammals, arthropods, plants, etc. (Sen et al. 2014, Rego et al. 2019). The density of the Actinobacterial population is determined by their habitat and the existing climate conditions. The effect of climate on the distribution of Actinobacteria was analyzed by Hiltner and Strömer 1903, who indicated that the soil microbial flora is more in the autumn season than in spring because of the available crop residues in this season. But, during the winter, frost reduces their abundance in the population. The class Actinobacteria contains 16 orders and the order *Actinomycetales* is limited to family *Actinomycetaceae*, and the other suborders that were part of this order are now considered as specific orders. So, 43 families within the phylum Actinobacteria are allocated to a single class, Actinobacteria, whereas the other five classes together are limited to10 families (Zhang et al. 2019, Barka et al. 2015). Various lifestyles are shown by *Actinobacteria*, and the phylum has pathogens (e.g. *Mycobacterium* sp. *Nocardia* sp. *Tropheryma* sp. *Corynebacterium* sp. and *Propionibacterium* sp.), soil-dwelling (*Streptomyces* sp.), plant commensals (*Leifsonia* sp.), symbiotic nitrogen-fixing (*Frankia*), and gut inhabiting (*Bifidobacterium*). Actinobacteria, are the source of various biocatalytic tools such as acid-producing *Corynebacteria*, secondary metabolite producing *Streptomyces*, carotenoid building *Micrococcus* strains, acid fermenting *Propionibacteria*, probiotic *Bifidobacterium*, xenobiotic *Gordonia*

species, and *Rhodococci,* etc. (Tischler et al. 2019). Most of the Actinobacteria spend the major part of their life cycles as semi-dormant spores and are saprophytic, soil-dwelling organisms and the phylum has adjusted to a wide range of ecological environments (Goodfellow & Williams 1983). This phylum is also known for its diversity in chromosome topology. Genus *Frankia*, *Salinispora* possess circular chromosomes but the major genera *Streptomyces, Rhodococcus* are having linear chromosomes and may or may not have plasmids. The linear plasmid pSLA2s was first identified in *Streptomyces rochei* (Hayakawa et al. 1979) which further led to the discovery of linear plasmids in other Actinobacteria. The size of the plasmids ranges from 12 to 600 kb in size (Kinashi et al. 1987; Sakaguchi 1990) and are mega-plasmids, and the size of linear chromosomes in Actinobacteria vary from 8 to 10 Mbp (Hopwood 2006). Though the genes involved in secondary metabolite production remain in clusters on the chromosome (Hopwood 2006), studies have also identified biosynthetic clusters on the large linear plasmids (Novakova et al. 2013). Nowadays, its members are considered among the most effective settlers of all environments in the extremobiosphere, in contrast to the belief that Actinobacteria are autochthonous soil and freshwater organisms (Bull et al. 2011).

Thermophiles

The Actinobacteria which grow at high temperatures ranging from 40 to 80° are termed as thermophilic organisms (Tortora et al. 2007). They are observed in moldy hay (Corbaz et al. 1963), self-heating plant residues, cereal grains, sugar cane bagasse (Suihko et al. 2006), decaying vegetable materials, and compost heaps (Henssen & Schnepf 1967). These may be obligate thermophilic or moderately thermophilic Actinobacteria. The obligate thermophilic can grow in the temperature range between 37 and 65 ° C, but optimum growth takes place at 55–60 °C. The moderately thermophilic Actinobacteria grow at 28–60 °C and require 45–55 °C for optimum proliferation (Jiang & Xu 1993). Whereas thermotolerant Actinobacteria can thrive at temperatures up to 50 °C

(Lengeler et al. 1999). Some examples of thermophilic Actinobacteria are *Amycolatopsis ruanii* and *Amycolatopsis thermalba.* The ability to grow in high temperatures is due to the electrostatic, hydrophobic and disulfide bonds in the proteins of these organisms (Ladenstein and Ren 2006). The presence of special proteins known as chaperones (Singh et al. 2010) and other proteins that bind to DNA and inhibit their denaturation at high temperatures.

Psychrophiles

Psychrophiles are the cold-loving organisms and most copious organisms on earth in terms of its diversity and distribution (Margesin et al. 2008). Actinobacteria phylum has been considered as one of the most prominent microbial divisions in different Antarctic regions (Cary et al. 2010; Pearce et al. 2012). This phylum is recognized as a producer of a wide range of secondary metabolites with different activities including herbicides, antifungals, antitumor or immunosuppressant compounds, and anthelmintic agents (Manivasagan et al. 2014). It has been shown that old permafrost has more amount of Actinobacteria (Willerslev et al. 2004). Antarctic Actinobacteria isolates belonging to the genus *Arthrobacter, Streptomyces,* and *Rhodococcus* exhibited antifungal activities (Santos et al. 2020). The genus *Arthrobacter* was reported from alpine permafrost in China (Bai et al. 2006). The psychrophilic and psychrotolerant Actinobacteria of *Nocardiopsis and Streptomyces* were isolated from the water samples of the Polar Frontal region of the Southern Ocean (Sivasankar et al. 2018). The psychrophilic Actinobacterial isolates are able to grow at low temperatures and alkaline conditions, produce a variety of enzymes such as proteases, amylases and cellulases (Zhang et al. 2007). Psychrophiles are subjected to temperature fluctuations and frequent freeze-thaw events. This has led to the evolution of several adaptation mechanisms concerning reproduction, metabolic activities, and survival and protection strategies in these organisms. Culture dependent and culture-independent molecular methods and the advancing fields of genome and proteome analyses will reveal more

about psychrophilic lifestyle (Margesina & Miteva 2011)

Xerophiles

Desert soil is also designated as an extreme terrestrial environment and organisms growing in this extreme environment are designated as xerophiles. The distribution of Actinobacteria in sandy soil (Cario, Egypt; Falmouth, MA), black alkaline soil (Karnataka, India), sandy loam soil (Keffi Metropolis, Nigeria; Presque Isle, PA), alkaline desert soil (Wadi El Natrun, Egypt; Wadi Araba, Egypt), and subtropical desert soil (Thar, Rajasthan), are established from different studies and *Streptomyces sp*. were found dominant followed by *Nocardia, Nocardiopsis,* and *Actinobacteria* (Cundell & Piechoski 2016). The isolates recovered from a desert soil sample collected in Beni-Abbes (southwest Algeria) were named Nonomuraea sp. (Badji et al. 2007) *Streptomyces youssoufiensis* sp. nov., was identified from Moroccan phosphate mine by Hamdali et al. 2011. In 2019, Nafis et al. reported the isolation of different Actinobacterial genera (*Streptomyces, Nocardioides, Saccharomonospora, Actinomadura*, and *Prauserella)* from Moroccan desert soil of Merzouga, Draa sfar mining sites which exhibits plant growth-promoting activities. The Actinobacterial isolates from the Algerian Saharan desert (Badji et al. 2006), Atacama Desert (Rateb et al. 2011), Egyptian desert (Koberl et al. 2011), Qinghai-Tibet Plateau (Ding et al. 2013), and Thar desert (Thumar et al. 2010) were studied for bioactive metabolites. The chloramphenicol was the first antibiotic isolated from Saccharothrix sp. PAL54A strain from Saharan soil in Ghardaïa (Aouiche et al. 2012). Currently, the focus has been diverted to extremophilic Actinobacteria with the anticipation that these organisms would add a novel arena to antimicrobial product research (Zitouni et al. 2004; Dhanasekaran et al. 2014).

Man is always curious to know about the existence of life on another planet such as Mars. This has led to the establishment of the Mars Desert Research Station (MDRS) where astrobiology research has been undertaken. MDRS in southeast Utah is situated in a cold arid desert where the

conditions are comparable to those on Mars. The microbial flora of this terrestrial Mars analog revealed several extremophilic Actinobacteria similar to uncultured Actinobacteria of the cold desert of McMurdo Dry Valleys, Antarctica and methane hydrate-bearing deep subsurface marine sediments in Nankai Trough (Japan). Most of them mainly belong to order Acidimicrobiales (Xu et al. 2018) thus indicative of putative xerophilic micro-organisms. Others were related to *Sporichthya* sp. from an ice core and is psychrophilic in nature (Direito et al. 2011)

Hydrophiles

Actinobacteria are widely distributed in aquatic habitats both fresh and marine environment. The major Actinobacteria dwelling in freshwater include *Actinoplanes, Micromonospora, Rhodococcus, Streptomyces,* and endospore-forming *Thermoactinomyces* (Cross et al. *1*981). The common freshwater Actinobacteria and found to be indigenous to such habitats is *Micromonospora* where they turnover complex sugars such as cellulose, chitin, and lignin.

Halophiles-Marine and Mangrove ecosystem

Actinobacteria is part of the marine microbial community of sediment samples that originated from terrestrial habitats and were disseminated to the sea in the form of resistant spores (Goodfellow & Haynes 1984). The first marine Actinomycete species to be characterized is *Rhodococcus marinonascene* (Helmke & Weyland 1984) followed by *Dietzia, Rhodococcus, Streptomyces, Salinispora, Marinophilus, Solwaraspora, Salinibacterium, Aeromicrobium marinum, Williamsia maris,* and *Verrucosispora* (Jenson et al. 2004). Grossart et al. (2004) have reported that Actinobacteria is the major phylum colonizing marine organic aggregates which helps in the disintegration and mineralization of organic matter (Magarvey et al. 2004). Recent studies established the presence of indigenous marine Actinobacteria in the oceans and in different marine habitats (Stach et al. *2*004). Marine Actinobacteria from sponges was isolated using nutrient supplements and

enzymes have been reported (Kim et al. 2005). The discovery of some strains that display specific marine adaptations and others that are metabolically active in marine sediments was described (Jensen et al. 2008). The culture-dependent studies have shown the existence of indigenous Actinobacteria in the oceans. Various marine Actinobacteria, such as *Dietzia maris, Rhodococcus erythropolis,* and *Kocuria erythromyxa,* from a subseafloor sediment core collected at a depth of 1225 meters off Hokkaido was isolated by Innagaki et al. (2003). Actinobacteria from soil samples belonging to the salt pan regions of Cuddalore, Parangipettai, India was isolated and screened for primary antibacterial activity by Dhanasekaran et al. (2005). *Streptomyces* sp. and *Saccharomonospora* sp. showed promising antimicrobial activity against different bacteria. The marine environment is a less exploited source of Actinobacterial diversity and their metabolites. Compared to the terrestrial microflora, marine Actinobacteria growing in the extremely saline environment produce different types of metabolites (Cross 1982). They thrive under high pressure and anaerobic conditions at temperatures just below 0 - 8 °C on the deep seafloor to high acidic conditions at temperatures of over 8 - 100 °C at the mid-ocean folds (Stach et al. 2005). This is reflected in the wide genetic and metabolic diversity shown by marine Actinobacteria (Bull et al. 2005).

There has been a growing interest in marine water habitat as a source of Actinobacteria that produce beneficial metabolic products that are known to be the producers of half of the discovered bioactive secondary metabolites (Berdy 2005), which may be antitumor agents (Cragg & Newman 2009) notably antibiotics, immunosuppressive agents (Mann 2001) and enzymes (Hill and Prins 2016). So it is evident that Actinobacteria is an important source of biologically active compounds and it is highly unexplored. (Lee et al. 2014). Though several studies have been conducted in this area it is far inconclusive. Exploration of a wide range of Actinobacteria communities in the marine environment will definitely answer the quest for new metabolites in the future.

Mangrove forests are large ecosystems prevalent in tropics and subtropics; they make up over a quarter of the total coastline in the World (Saddhe et al. 2016). Mangroves form the transient ecosystem which exhibits the edge effect and shows wide species richness. They provide habitat for different flora and fauna and also a treasure trove for the microbial biome. Since it is an ecotone habit, it is rich in biodiversity. It is a link between land and sea which serves as breeding and nursing grounds for aquatic organisms. This ecosystem is an active geographical zone that is rich in organic sediments remains the homeland of microbes. The mangrove ecosystem is saline and highly rich in organic matter and an untapped source for screening and isolation of potential bioactive metabolites (Newman & Cragg 2007). These bioactive compounds have a unique structure and chemical features which is not found in natural terrestrial products (Kathiresan & Bingham 2001). The microbes in mangrove habitats not only produce primary and secondary metabolites but are also involved in an important ecological role in soil organic matter decomposition and mineralization (Ghosh et al. 2011). Actinobacterial species such as *Streptomyces, Micromonospora,* and *Nocardioform* were found to be abundant, in the anaerobic mangrove rhizosphere, which is 1000 to 10000 times smaller than the aerobic population because of tidal influence (Tan & Cao 2009). *Nocardia* isolated from mangrove habitat produced metabolites that strongly suppressed human cell lines, such as gastric adenocarcinoma (Schneider 2009). Actinobacteria play an active role among the mangrove bacterial communities, because of their divergence and capacity to synthesize chemical compounds of high economic value (Watve et al. 1999) Actinobacteria participate in many important biochemical processes in the soil. From the marine ecosystem *Actinomyces, Actinopolispora, Micromonospora, Micropolispora, Nocardia, Rhodococcus*, *Streptomyces*, *Streptosporangium* and *Streptoerticillium* are reported so far (Lechevalier & Lechevalier 1970). Several studies indicate the importance of Actinobacteria in antibiotic metabolite production. The metabolites

are used as anticancer compounds, antifouling compounds, bioremediation, PGPR, immunomodulators, etc (Singh & Dubey 2018). Studies on the biodiversity of Actinobacteria from the mangrove ecosystem are important for biotechnological exploitation. Actinobacteria are well known for the production of commercially important bioactive compounds and antitumor agents in addition to enzymes of industrial interest (Khattab 2017). It has been shown that approximately 203 of the naturally occurring antibiotics are from Actinobacteria (Takizawa et al. 1993). Actinobacterial community, which resides in mangrove sediments, are poorly studied (Sivakumar 2001). Currently, enrichment techniques, new selection methods have led to the isolation of novel Actinobacteria from sediment samples (Jensen & Lauro 2008). There is a huge prospect for the isolation of novel secondary metabolites from Actinobacteria of mangrove habitat. The metabolites from these Actinobacteria possess distinct chemical structures that may lead to the synthesis of new drugs that could be used against resistant pathogens (Stach et al. 2004)

Acidophiles

The proliferation of *Actinobacteria* in the soil is preferred by low humidity, especially when the spores are immersed in water. The growth is very limited and may be halted in dry soils (Kim et al. 2003). They prefer soils with a neutral pH. and grow well in soil with pH between 6 and 9. But there are reports for the isolation of some strains of *Streptomyces* from acidic soils (pH 3. 5) (Kim et al. 2003) and acidic forest and mine drainage soil, that grow in the pH range from about 3.5 to 6.5. *Streptacidiphilus anmyonensis* sp. nov., *Streptacidiphilus rugosus* sp. nov. and *Streptacidiphilus melanogenes sp.* nov. (Cho et al. 2008) grow well in acidic soil. The acidophilic Actinobacterium is an important group to study the novel bioactive metabolites. This is evident from the genome-based studies conducted on *Streptomyces yeochonensis* CN732 and the presence of cysteine transpeptidases among the Biosynthetic Gene Clusters (BGC) which indicates their role in the biosynthesis of secondary metabolites

(Malik et al. 2020). The acidophilic Actinobacteria are considered as the sources of polyketides such as polyether ionophores that exhibit antagonistic activity against drug-resistant bacteria and parasites (Wang et al. 2011).

Lithophile (stone dwelling)

Several reports for stone-dwelling Actinobacteria are available recently which shows growing interest and suggests more studies in this arena. Actinocommunity is known for its role in ecological succession as one of the pioneer communities. The major family which has been isolated from the stone niche is *Geodermatophilaceae*, an Actinobacterial family (Sghaier 2016) which is endemic to soil (Sen et al. 2014) that consists of three genera: *Geodermatophilus, Blastococcus* and *Modestobacter* that was isolated from desert soils (Luedemann 1968), seawater (Ahrens & Moll 1970) and Antarctic regolith (Mevs et al. 2000), respectively. The omnipresence of *Geodermatophilaceae* in different biotopes including prominent rocks (Eppard et al. 1996) and desert sandy soils (Montero-Calasanz et al. 2012) and its evolutionary ability is intriguing. The soil and stone niches have provided us knowledge regarding the wide distribution of *Geodermatophilaceae* (Gtari et al. 2012; Normand et al. 2012), and has created interest in their evolutionary and adaptation mechanisms to harsh environments.

Cave dwelling Actinobacteria

Caves are seldom explored and is prevalent with different mineral structures, permafrost and previously unknown organisms that have evolved in a microenvironment with more or less constant temperature, humidity, air composition and other conditions over long periods (Culver & Sket 2000). These biomes are of great interest due to the presence of microorganisms, which have been subjected to evolution in stable conditions for a long duration (Grady 2005). Also, caves are contained zones with limited resources and have little energy exchange with the environment. Maciejewska et al. (2015) isolated the strain *Streptomyces lunaelactis* sp. nov. from moon milk speleothem collected in the cave

'Grotte des Collemboles' (Comblain-au-Pont, Belgium)

The novel species was isolated from caves and cave-related habitats and were from the genus *Streptomyces*, *Amycolatopsis* and *Nocardia*. *Antricoccus, Beutenbergia, Knoellia, Lysinibacter Spelaeicoccus* and *Sphaerimonospora* are the novel genera isolated from cave soils. The genus *Hoyosella* was isolated from the biofilm on the ceiling and wall of Altamira cave, Spain (Jurado et al. 2009). The cave environment which is extremophilic in nature, poses pressure for the inhabitant microorganisms at the genomic level, resulting in the evolution of new species and their production of more metabolites (Tiwari & Gupta 2013). Therefore, caves are considered an exquisite ecosystem for the identification of new Actinobacteria.

Another interesting aspect is the diversity of Actinobacteria in beehives in Thailand was studied by Promnuan et al. (2009). They had isolated and identified thirty-two isolates using morphological, physiological, chemical and molecular characterization. The major isolates have belonged to the genera *Streptomyces, Nonomuraea, Nocardiopsis* and *Actinomadura*.

2.4 Bioactive metabolites

Bioactive metabolites are compounds that originated from living organisms that affect the different activities of living cells. The term "bioactive" is comprised of two words: bio- and -active. Bio- from the Greek (βío-) "bios" [bio-, -bio], refers to life and –active from the Latin "activus", means: dynamic, full of energy, with energy or involves an activity (Bernard & Dromard 2011). This activity represents all phenomena of life, a functioning or a process. These effects may be progressive or deleterious depending on the substance, the dose or the bioavailability.

Out of the 22500 microbial metabolites discovered so far, about 17% (3800) are from unicellular bacteria *Bacillus* spp. and *Pseudomonas* spp.); 45% (10 100) are products of Actinobacterial origin; and about 38% (8, 600) are from fungi (Demain & Sanchez 2009). Amidst the filamentous Actinobacteria, approximately 75% (7600) of metabolites are produced by species of

the genus *Streptomyces* (Berdy J. 2005; Lam 2007). 140 Actinobacteria genera have been described to date. Genus *Streptomyces* alone produces a large number of bioactive molecules. The genera *Saccharopolyspora*, *Amycolatopsis, Micromonospora* and *Actinoplanes* produce bioactive metabolites but at a lower scale compared to *Streptomyces* (Solanki et al. 2008). It has great biosynthetic potential that remains unopposed without a possible competitor among other microbial groups. Another genus *Arthrobacter* spp. exhibit great metabolic versatility and are able to degrade pollutants and xenobiotics such as heavy metals (As, Cd, Cr, Hg). But the prospect of finding highly potential Actinobacteria from terrestrial habitats is reduced due to the wide exploitation for antibiotic production. So, attention has been diverted to unexploited and extremophilic habitats.

Human activities are creating new compounds and the microbial community is constantly evolving to cope up with this stress. The mechanism of action of the microbial community in vitro and in vivo conditions may deduce the remedy for degradation of the environment as well as the drug resistance in organisms. The metabolic pathways involved in xenobiotic degradation is not yet studied in a comprehensive manner (Maurice et al. 2013). The detailed study of the xenobiotic activity of pathogenic and nonpathogenic Actinobacteria in different niches may help us to understand the intricate mechanism of microbial community with the environment. It may open up new avenues of understanding that could have many applications in fields diverse as agriculture, biotechnology, ecosystem monitoring. Integrated pest management utilizing microbial pesticides is gathering momentum. This is because they are more specific, have low relative cost and are more eco-friendly (Kesho,2020). Many reports indicated the important role played by Actinobacteria in the management of *Spodopetra littoralis* (Bream et al. 2001), *S. litura* (Arasu et al. 2013), *Musca domestica* (Ghazal et al. 2001), *Culex quinquefasciatus* (Khawagh et al. 2011), *Drosophila melanogaster* (Gadelhak et al. 2005), *Helicover paarmigera* (Bapatla et al. 2021),

Anopheles mosquito larvae (Dhanasekaran et al. 2010). Among the biological control agents derived from different microbes, Actinobacteria especially *Streptomyces* sp. are some of the most important microbial resources which can provide potential new bioactive compounds for use as insect-control agents (Kaur et al. 2016).

2.5 Streptomyces genera

The most widely distributed genus of Actinobacteria, Streptomyces has been the focus of research by biologists because of the commercial applicability of the substances produced.*Streptomyces are* mainly soil -dwelling saprophytes having a large genome size of 6-12 Mbp (Tidjani et al. 2019) *S. cavourensis* is a producer of the antibiotic chromomycin and *S. michiganensis* is involved in the synthesis of anthelmintic acid and antiprotozoal substances (Silva et al. 2013).

Streptomyces genus of Actinobacteria has been known for its prolific production of metabolites which may help to combat antibiotic-resistant pathogens (Berdy 2005; Jones & Elliot 2017). Around 13, 700 bioactive secondary metabolites are produced from Actinobacteria in which *Streptomyces* spp. alone comprised approximately 10, 400 (75%) and (39%) of entire microbial products (Sousa et al. 2016). Notably many such secondary metabolites are potent antibiotics, a trait that has turned *Streptomyces* spp. into the primary antibiotic-producing organism exploited by the pharmaceutical industry (Atta et al. 2015).An orally active metabolite that exhibits immunosuppressive effect Everolimus is a derivative of rapamycin, originally produced by the actinomycete *Streptomyces hygroscopoicus* (Chapman & Perry 2004). A cyclic lipopeptide, Daptomycin which possesses good antibacterial activity and has been approved for the treatment of complicated skin infections was produced from *Streptomyces roseosporus*. "Delta-Indomycinone: a new Member of Pluramycin class of antibiotics from marine Actinobacteria and "Himalomycin A and B were isolated from marine *Streptomyces* sp. (Maskey et al. 2003). Studies have revealed that in spite of the occurrence

of genes that are present in antibiotic synthesis in the genome, Actinobacteria fail to exhibit antagonistic properties *in vitro.* Identification of natural conditions for the expression of dormant genes is crucial for the screening and isolation of novel antibiotics (Trenozhnikova & Azizan 2018).

Streptomyces pluripotens MUSC 137 isolated from mangrove soil in Malaysia shows antioxidative and cytotoxic activities were established by Ser et al. 2015. Similarly, antimicrobial and cytotoxic Activity of marine *Streptomyces parvulus* VITJS11 crude extract was reported by Naine et al. 2015. Bream et al. showed potent biological activity of secondary metabolites of Actinobacteria such as *Streptomyces* and *Streptoverticillum* against *S. littoralis* which caused larval and pupal mortality. Several metabolites from genus *Streptomyces,* such as avermectin, prasinons, doramectin, milbemycin, nanchangmycin, dianemycin and spinosad have been established as potential protective agents against a variety of insect pests and are friendly to the environment (Omura 2008). These microbial pesticides offer an alternative to chemical insecticides with increased target specificity and ecological safety so that they are used either uniquely or in combination with other pest management programs.

Actinobacteria have been reported to produce an extensive range of prospective commercial enzymes that can be used in biotechnological applications and pharmaceutical fields. Advances in sequencing technology and bioinformatics field have made it possible to study microbial enzyme production by using proteomics and transcriptomics (Pieper 2005). Amylases from *Streptomyces* sp. has an important role in biotechnological applications in different industries. Pectinases are the enzymes used in the food industry which had been isolated from different species of *Streptomyces* such as *S. lydicus* (Jacob et al. 2008). They find application in extraction and clarification of wines, juices, oils, flavoring compounds and preparation of linen fabrics and hemp manufacture (Horikoshi 1999). *Streptomyces* are the main producers of xylanases in the Actinobacterial phylum. Xylan is the most important component of

hemicelluloses and it is used in the improvement of the pulp and bio bleaching industry (Ghorbel & Prakash 2012). Enzymes from the Actinobacterial population find wide application in different areas of industry and are emerging as a potent source of novel enzymes. Pigments are industrially important as they are used for coloring textiles, cosmetics, etc. Efforts have been taken to replace synthetic colors with natural sources since the former has several harmful effects. Numerous pigments are synthesized by Actinobacteria using natural and artificial media. Subhash and Kulkarni (2015) could obtain red color melanin by *Streptomyces bikiniensis* using a tyrosine medium and used it as an antimicrobial agent. *Streptomyces torulosus* produces three different dyes with various colors and were used for dyeing wool and polyamide fabrics (Kheiralla et al. 2016). Recently, bio-cosmetics are pushing through the cosmetics industry in the world and the demand for biocosmetics is rising and quite significant. Screening and isolation of Actinobacteria from underexplored habitat may definitely boost the cosmetics and pharmaceutical industries in the future.

The phylum Actinobacteria with black pigments melanin is involved in the cycling of organic compounds and also in the production of soil humic acid (Schaeffer et al. 2015).It has also been reported that mixed cultures are more effective in bioremediation in comparable to pure cultures (Joutey et al. 2013).The production of various bioactive compounds has been identified however, specific metabolic pathways of synthesis and their regulation remains unexplored.

2.6 Characterization

Soil microbes are greatly important in agroecosystem, the microbial community structure assessment with accurate and reliable methodology is necessary. The methods for studying soil microbial diversity can be categorized into microscopy, biochemical-based and molecular-based methods (Kirk et al. 2004). The transmission electron microscope studies have provided much useful information about the structure of organisms belonging to various genera of the Actinobacteria Details of the

internal, vegetative and reproductive structures of several genera have been obtained from the examination of ultra-thin sections. The development of spores in sporangia-forming genera have been given by Rancourt & Lechevalier (1963) who studied *Microellobospora.,* Lechevalier & Holbert (1965) who examined *Actinoplanes* and Lechevalier & Holbert (1966) who studied the genera *Streptosporangium, Spirillospora* and *Actinoplanes.*

Gas chromatography-mass spectrometry (GC-MS) is a hybrid analytical technique that couples the separation capabilities of GC with the detection properties of MS to provide higher efficiency of sample analyses. While GC can separate volatile components in a sample, MS helps fragment the components and identify them on the basis of their mass. GC-MS provides enhanced sample identification, higher sensitivity, an increased range of analyzable samples, and faster results, which enable a whole new range of applications for GC-MS in several areas (Sahil et.al. 2011). Microbial extracts can be subjected to GC-MS for identifying and characterizing the bioactive compounds.

2.7 Tea Industry

Tea industry is one of the oldest industries in India. In India the four main tea-producing states are Assam, West Bengal, Kerala and Tamil Nadu. Tea is also grown in parts of Tripura and Himachal Pradesh. There are more than one million workers in the tea industry in India comprising mainly of scheduled castes, tribes and ethnic minorities of which more than 50% are women. (CEC report http://www.cec-india.org)

The Darjeeling hills and Dooars region of West Bengal have 283 tea gardens, employing 350, 000 permanent and casual workers, who earn Rs 176 per day besides the weekly ration. Sprawling across the districts of Darjeeling, Jalpaiguri and Alipurduars, the belt has another 40, 000 small growers who employ one lakh laborers. 1,86, 559 families residing in the tea estates of hill, Terai and Dooars areas of North Bengal. Chengmari T.E. has the highest no. of families i.e. 4, 950 whereas Girish Chandra T.E. has the lowest no. of families i.e. 05. Out of

283 Tea Estates, only 166 Tea Estates have hospitals. Out of these 166 Tea Estates only 56 Tea Estates have full time residential doctors. Other 110 Tea Estates hospitals depend on visiting doctors (Ghosh 2016). The five major health afflictions were identified among the estate workers. They are continuous cough/dry cough, low blood pressure/high blood pressure neck pain/shoulder pain, Respiratory and skin problems, Continuous Cough/ result of infection by cold and flu viruses.

Tea is one of the major plantation crops in the world. Tea plants are subjected to attack by many pests species and reduce productivity which affects the economy negatively. According to Chen & Chen (1989) 1034 species of arthropods, 82 species of nematodes 1 algal and 350 fungal diseases are associated with tea plants globally. At present, India is the largest producer of pesticides in Asia and ranks twelfth in the world for the use of pesticides with an annual production of 90, 000 tons (www.teri.res.in/ pesticide.htm). Pesticides are poisonous substances and they are to be handled with extreme care. These are not easily degradable; they remain in soils, leach to groundwater and pollute the environment to a wide extent. These enter bodies of organisms, bio concentrates in the food chains and have an adverse impact on human health. The tea farmers have been applying different pesticides to reduce pest incidence in their tea gardens. Farmers are practicing cocktail preparation of two or more than two pesticides without knowing their compatibility, which is of serious concern to health and environment (Jallow et al. 2017). Again, based on the types of pesticides used, various signs and symptoms of diseases/ disorders have been observed among the tea growers and the relative risk also observed to be high (Shreshta & Thapa 2015) Absence of adequate protective measures were noticed that have increased the declining state of the health of farmers. To reduce the pesticide environment pollution and MRLs on tea product, safer pesticides as a last resort have to be considered (Gurusubramanian et al. 2008). Recently, microbial and botanical bio-pesticides have gained popularity against different insect pests of various

crops. Among the biological control agents derived from different microbes, Actinobacteria especially *Streptomyces* sp. are one of the most important microbial resources which can provide potential new bioactive compounds for use as an insect-control agent. Many reports indicated the important role played by Actinobacteria and their secondary metabolites in the management of *Spodopetra littoralis, Culex quinquefasciatus, Helicoverpa armigera* (Yandigeri 2021).

Among biological approaches, the use of microbes with degradative ability is considered the most efficient and cost-effective option to clean pesticide-contaminated sites (Massiha et.al 2011). Several groups of Actinobacteria are capable of removing heavy metals from polluted environments.

2.8 Bioinformatic studies.

Bioinformatics tools become relevant in this aspect. The language of DNA which is the four-letter alphabet that is expressed as a triplet codon for the amino acid is the key for fundamental gene expression. The degeneracy of codons for 18 amino acids except methionine and tryptophan showed that different sequences of DNA produce identical protein sequences (Knight et al. 2001). The degeneracy mainly occurred in the third position of the codon. Data from whole-genome sequences help to study the preference of codons among organisms. It has been noticed that variation of choice of codons to represent amino acids is not only observed among species from the different taxonomic group, but also showed significant variation among individuals of the same species, across different genes in the same genome and even across regions in the same gene (Sharp et al. 2005). But, the codon bias is most prominent in species from different taxonomic groups even in proteins with identical functions. This phenomenon of species-specific codon choice is known as “codon dialect” which signifies the codon-usage bias observed across different organisms (Ikemura 1985).

CODON USAGE

The codon usage pattern is a unique feature of a particular organism. It helps us to understand the gene expression,

horizontal gene transfer and also enables to determine phylogenetic relationships between organisms. The study of codon usage (Zhu et al. 2008) patterns of several genes and genomes is a popular technique to characterize and analyze genomic trends from a bioinformatics-based perspective. Codon usage patterns and preferences vary significantly within and between organisms (Sen et al. 2007; Sharp et al. 1987).

CODON W software developed by Pedan in 1999 became very popular and widely used for studying codon usage and multivariate analysis because of its error-free analysis. The parameters such as GC content (amount of guanine-cytosine in the nucleotide sequences), GC3 content (frequency of either G or C nucleotides in the third position of synonymous codon), the effective number of codons used in a gene (Nc), frequency of optimal codons (Fop), CBI (codon bias index), GRAVY (hydrophobicity of amino acids) are included in this analysis. The most obvious factor that determines codon usage is mutational bias that shapes genome GC composition. Mutational bias is responsible not only for intergenetic difference in codon usage but also for codon usage bias within the same genome (Ermolaeva 2001). Most of the organisms with a balanced AT/GC genome have codon heterogeneity (Sen et al. 2007). Highly expressed genes contain a higher percentage of codons that are transnationally optimal (Ikemura 1985). Codon heterogeneity in the genome can be studied by GC content, GC 3 content, effective number of codons (Nc). Nc measures the overall codon bias of synonymous codons (Wright 1990). It ranges from 20 (in the case of one codon for one amino acid) to 61 (where all codons are used). The GC content estimates the amount of the guanine-cytosine in the nucleotide sequences. The GC3 infers the frequency of either G orC nucleotides present in the third position of the synonymous codon. This is not applicable to methionine, tryptophan and the termination codon. These values have a direct correlation with Nc. Its measures the synonymous codon usage of genes and its value ranges from 20-62 (Peden,1999).

The frequency of optimal codons *(Fop)* is the fraction of synonymous codons

which are optimally used. It is given by (*Fop) =Noc/ Nsc* where N represents the frequency of each codon, *Noc* and *Nsc* represents optimal codons and synonymous codons respectively. The *Fop* values range from 0 to 1. If the value of *Fop* is 1, it shows the usage of all optimal codons.

GRAVY scores determine the hydropathic indices of amino acids (Kyte & Doolittle 1982). A positive score indicates the hydrophobic nature and a negative score shows the hydrophilic nature of amino acids.

The Relative Synonymous Codon Usage (RSCU) values for the genes are calculated to understand the characteristics of synonymous codon usage without the confounding influence of amino acid composition of different gene sample (Sharp & Li 1986). The codons with RSCU values >1.0 have positive codon usage bias (abundant codons), while those with RSCU values <1.0 have negative codon usage bias (less-abundant codons); and when the RSCU values are 1.0, it means that these codons are chosen equally or randomly, indicates lack of bias (Tsai et al. 2007). The RSCU is the observed frequency of a codon divided by the frequency expected if all synonymous codons for that amino acid are used equally. The synonymous codons with RSCU more than 1.6 were thought to be over-represented, while the synonymous codons with RSCU less than 0.6 were regarded as under-represented (Wong et al. 2010). The RSCU values are particularly useful in comparing codon usage between genes that differ in size and amino acid composition.

Codon adaptation index is a widely used index for studying gene expression in general and the efficiency of translation in particular. CAI has been used extensively in biological research. It has been used to study functional conservation of gene expression across different microbial species (Lithwick & Margalit 2005), to predict protein production (Futcher et al. 1999; Gygi et al. 1999), and to optimize DNA vaccines (Ruiz et al. 2006). CAI has recently been used for detecting lateral gene transfer (Bodilis

& Barray 2006).

The CAI program in EMBOSS (Rice et al. 2000), typically referred to as the EMBOSS.cai program is most popularly used. Another software for computing CAI is the web application called CAI Calculator 2 (Wu et al. 2005). The improved CAI is implemented as a new function in DAMBE (Xia & Xie 2001, freely available at http:// dambe.bio.uottawa.ca/dambe.asp), which uses a windowed user interface. DAMBE can read 20 standard sequence file-formats including files in the simple FASTA format and the more involved GenBank format or trace files from automatic sequencers. The CAI function can be accessed by clicking 'Seq. Analysis Codon usage'. The ensuing dialog box is self-explanatory, except that, for species without a reference set of highly expressed genes, a codon table based on tRNA anticodon can be used by clicking the alternative option button.

CAI values vary from 0 to 1 and higher CAI values indicate that gene of interest has a codon usage pattern more similar to the highly expressed genes (Sharp & Li 1987).

Codon usage bias (CUB) is usually defined as a species-specific deviation from uniform codon usage in the coding regions of genomic sequences. This bias is possible due to the redundancy of the genetic code, which allows differential use of synonymous codons (Behura & Severson,2013). The particular pattern of bias observed in a given species is thought to be the product of drift and selection pressures acting on a number of parameters, but mainly on tRNA gene copy number and genomic % GC content. CUB is therefore a strong species-specific statistic with numerous applications, such as gene prediction or the identification of laterally transferred genes.

Protein energetic cost:

It can be defined as the energy consumed for the synthesis of an amino acid encoded by a specific functional codon. Mostly, the energy cost of potentially highly expressed genes is lower than the energy budget of the rest of the proteome. But it cannot be applied to all organisms. In the case of Actinobacteria, it has been shown that

energy cost varies with its niche (Sarkar et. al 2018)

CAI is a major index to measure the m RNA expression level. Generally, genes with 10% of the highest and lowest CAI value are chosen as potentially highly expressed and potentially lowly expressed genes respectively. The remaining genes are considered as potentially medially expressed genes. DAMBE software calculates the EC (Dambe ver. 6.4.81). The EC values can be analyzed statistically using ANOVA test, F-test and t-test. Heat maps are generated using R statistical software (Kim 2019).

2.9 Metabarcoding analysis

We are grossly ignorant of bacterial life on earth. Environmental microbiologists estimate that less than 2% of bacteria can be cultured in the laboratory (Wade 2002). In the mouth, we do rather better, with about 50% of the oral microflora being culturable. For other body sites, the figure is unknown but is likely to be similar to that found in the mouth or higher. For example, the colonic microflora is suspected to be predominantly uncultivable. It is therefore likely on numerical grounds alone that uncultivable and therefore uncharacterized organisms are responsible for several oral and other human infections. The best example is syphilis, caused by the spirochete *Treponema pallidum*, which remains unculturable (Radolf et al. 2016).

Most of the microbes don't grow under *in vitro* conditions. The proportion of uncultivable microbes to cultivable microbes is still very high(Nichols et al. 2010) To circumvent such cultivation limitations, strategies have been developed based on the extraction of microbial DNA directly from an environmental sample and its subsequent analysis or exploitation (for biotechnological purposes) independent of its original host. This approach, which is based on the recovery of a sample's microbial metagenome (the sum of all microbial genomes), has, in theory, great potential for biotechnological and ecological studies of the system under investigation, for example, soil(Lombard et al. 2011). It is recognized as the best option to access the microbial genetic diversity present. Such a metagenomics-based

view of the community will aid in enhancing our understanding of microbial functioning and interactions in a soil ecosystem. Shotgun metagenomic sequencing has enabled to detect and characterize the diversity and function of the microbial communities. This technology will help to discover new microbial products as well as new species. The metagenomic study involve several steps starting with the extraction of DNA from the source, library construction, sequencing, data analysis etc. Data screening can be done by either sequence based or by function driven analysis. The specifically designed primers or probes based on the sequences of previously identified bioactive compounds are used in sequence driven analysis. These primers are used for PCR amplification of the metaDNA and subsequently sequenced and cloned to the expression vectors. Contrastingly, in the function driven analysis aids us to identify novel compounds. Here in this method, DNA will be subjected to restriction digestion and a library of clones are prepared which will be further analysed for the production or synthesis of new molecules (Datta et al. 2020). It has potential for numerous industrial applications when used to isolate genes involved in the synthesis of new molecules such as antibiotics of the polyketide class by cloning of such genes into hosts that express them. The extraction of the metagenome and its sequencing will enable the physiological requirements of the dominating non-cultivated bacteria to be deciphered, which will help to determine the appropriate growth conditions for these recalcitrant bacteria (Lombard et al. 2011). This technology will also revolutionize the clinical diagnosis area. Though in the nascent stage, metagenome has the potential in the profiling of the resistomes from different environments (Sukhum et al. 2019).

Actinobacteria is the second most diverse group of microbes after Proteobacteria. Its diversity comprises its presence in biotopes of extreme habitats such as deserts, minefields, stones, deep sea beds, etc. Actinobacteria which is a primitive and prominent phylum among prokaryotes are distributed in a wide range of ecological niches. Thus, they establish a

substantial proportion of the telluric microflora which is of extensive interest to the scientific community. Some of the most important microbes include *Streptomyces* which is the biological warehouse of various antibiotics. *Streptomyces* genus of Actinobacteria has been known for its prolific production of metabolites which may help to combat antibiotic-resistant pathogens (Berdy 2005). However, specific metabolic pathways of synthesis and their regulation remain unexplored. During the last decade many genomes and their plasmids of Actinobacteria have been sequenced (13402 complete genomes, to date as per IMG database) and made available for data mining which may deliver access to several potential biocatalysts that await interpretation.

Yet, Actinobacterial research for the most part is rather recent and knowledge on many members is still elusive. This review is an attempt to give a comprehensive account of various applications of Actinobacteria especially from extremophilic habitats based on the knowledge available today. The utilization of new technologies for culture, identification and classification of microbial flora, makes the Actinobacterial research dynamic and promising. There is much more to be explored in this area. The research in this dimension will definitely provide immense information that will be highly beneficial to mankind in the future.■