REVIEW OF LITERATURE

CHAPTER 2
REVIEW OF LITERATURE

2.1 General morphology of *Actinomycetes*

Actinomycetes are aerobic bacteria that are gram-positive and are capable of producing spores. They are classified under Actinomycetales, which may be identified by mycelium development on both the substrate and air during every life cycle phase. The results of the DNA: rRNA combination study and 16S ribosomal cataloguing can be phylogenetically connected to the high (G+C) proportion/ratio found in their DNA (>55%) [57, 58]. Within the bacteria domain, 18 entire lineages have been described up until this point as having been discovered. [59] This taxonomic assemblage holds immense importance and is deemed one of the most crucial groups. The terminology "*actinomycetes*" originates from ancient Greek lexicons. "actis" signifies "a ray," and "mykes" means "fungus." Given the shared qualities of *actinomycetes* with bacteria and fungi, the terminology "*actinomycetes*" was assigned to this group [60]. In addition, they have a sufficient number of defining characteristics that allow us to classify them as members of the "Kingdom of bacteria." *Actinomycetes* can synthesise antibiotics, biologically beneficial compounds, and bioactive molecules in secondary metabolites. These drugs include immunosuppressants, antibiotics, antitumor agents, and enzymes. In addition, it is known that these metabolites possess activities that are antibacterial, antioxidant, antifungal, anticancer, neuritogenic, anti-algal, antimalarial, and anti-inflammatory [61, 62]. Antihelminthic activity is also associated with these metabolites. The research findings unveil diverse cycles of life that are distinct from other prokaryotes and have a crucial impact on the organic matter cycling in the soil environment of their habitation. As has been demonstrated, many bioactive secondary metabolites can be produced by *actinomycetes*; developing novel antibiotic and non-antibiotic guide molecules while screening secondary microbial metabolites is becoming increasingly crucial. Furthermore, actinobacteria, commonly called *actinomycetes*, are essential to human medicine, food manufacturing, and agricultural methodologies. The ability of these organisms to collaborate is the driving force behind this activity. Even though they have been studied for the past 150 years, this aspect of their biology has been largely overlooked. In humans, it is anticipated that actinobacteria will structure approximately one-third of the gut microbiota, most of which will be comprised of bifidobacterium species, which are beneficial for human wellness and nourishment [63]. In conclusion, isolating previously unknown groups of microorganisms that are also excellent producers of bioactive secondary metabolites is a crucial component of the drug innovation aspect of microbial extract-based drug discovery. There are still plenty of environmental niches that have yet to be cultivated. Research on this topic is required to discover a broader range of novel *actinomycetes*. In general, unusual strains of *actinomycetes* are responsible for producing various compounds. In addition to accomplishing this rationale, a concentrated and laborious effort can be put in to isolate and screen novel strains to discover new combinations [64].

2.2 *Actinomycetes* Ecology and Habitat

Actinomycetes constitute the predominant microbial population, and they have a structure resembling thread-like filaments. When cultivated, however, they take on the appearance of hyphae like fungi, and they are the organisms responsible for the "earthy" smell characteristic of freshly turned healthy soil [65]. The *actinomycetes* are classified as a non-ubiquitous assemblage of microorganisms extensively distributed in typical ecosystems throughout the globe [66]. They live in various habitats within the environment [67, 68]. They primarily reside within terrestrial soil environments, although observed in diverse aquatic ecosystems, such as marine sediment samples [69], yet from maximum depth Mariana Trench [70]. The substantiation of their existence in highly hostile environments, specifically in cryogenic zones, has been established through empirical observation and research [71]. For instance, soils were taken from Antarctica [72] and even desert soil [73]. Based on a proportional survey, it has been determined that *actinomycetes* are the predominant inhabitants in soil sources, with the highest concentration found in surface layers and a gradual decrease in concentration with further penetration. The traits of *actinomycete* strains are ubiquitous across all soil layers [74]. Other advancements are required in the understanding of actinomycete diversity in limestone habitats. However, specific investigations have been sanctioned on the microbial community of caves, particularly limestone caves [75, 76]. *Actinomycetes represent the most* extensively dispersed category of microorganisms in the natural environment. The emission of geosmin by soil *actinomycetes* is responsible for the distinctive scent that arises when precipitation occurs following a prolonged period of arid weather [77]. Streptomyces species are ubiquitous populations in natural environments and typically constitute a fundamental constituent of the entire actinomycete community. As per the extant literature review [78], the number of cited Streptomyces species exceeds 600. The isolation and cultivation of specific actinomycete genera, such as Microbispora, Catenuloplanes, Micromonospora, Amycolatopsis, Actinoplanes, Kineospora, and Nonomuraea and Dactylosporangium, can be pretty challenging due to their slow growth and are commonly referred to as "rare *actinomycetes*" [79].

Furthermore, it is noteworthy that *actinomycetes* commonly exhibit heterotrophic behaviour within their natural surroundings. While some come via parasitic or mutualistic relationships with other plants and animals, most are strict saprophytes. *Actinomycetes* are widely acknowledged to play a crucial role in nutrient cycling. These microorganisms are characterised by their aerobic nature, although some, such as *actinomycetes*, can also thrive in anaerobic conditions. This information is discussed in Chapter One, which includes an introduction and a literature review. Frankia, for example, needs particular growth media and temperature and humidity settings to thrive. Blood agar, starch casein agar, trypticase agar, nutrient agar, and brain heart infusion agar are commonly used bacteriological media in the laboratory and are suitable for growing various *actinomycetes*. The Sporo*actinomycetes*, on the other hand, need a particular press to ensure that the spores differentiate and that their pigment synthesis is maximised [80].

2.3 Life Cycle of *Actinomycetes*

The life cycle of *actinomycetes*, which includes spores, vegetative mycelia, and reproductive mycelia, is crucial and serves as an essential model for the formation of bacteria. They undergo profound morphological transformations that are directly related to their physiological differentiation. Due to the availability of the complete genome sequence, the examination of *actinomycetes* biology has relied on comprehensive studies of Streptomyces coelicolor's entire life cycle. [81]. Streptomyces coelicolor is widely acknowledged as a paradigmatic example of a multicellular prokaryote characterised by a sophisticated developmental program encompassing sporulation and apoptotic cell death. Upon spore germination, the proliferation of vegetative components gives rise to a mycelium comprising a branching network of hyphae that passes through a moist substrate via the elongation of sub-apical branches and hyphal tips. This is subsequently followed by reproductive development, frequently followed by the growth of filamentous aerial hyphae and ends with the formation of chains of spores with only one gene. This bacterium has a multicellular prokaryotic model because of its complex developmental life cycle, which includes apoptosis occurrences [82, 83]. The life cycle of Streptomycetes is intricate and falls under the category of neither multicellular nor unicellular organisms. It commences with spore germination, disseminating filaments into the solid medium, ultimately forming vegetative mycelium. A complex series of events characterise this process. [84]. subsequently, the vegetative mycelium undergoes a developmental process leading to the formation of sporophores—the structures mentioned above exhibit vertical growth towards the surface, culminating in generating aerial mycelium. Moreover, the polynucleated aerial form is caused by developing aerial mycelium, which undergoes spiral formation followed by filament partitioning. The ultimate coverings transform into reproductive units known as spores, thereby initiating a recurring cycle. Microbial secondary metabolites comprise predominantly organic compounds, such as toxic substances, antibiotics, pigments, enzymes, and antitumor agents. These do not directly participate in the typical growth, development, or replication of the developing microorganism processes [85]. In contrast, microorganisms synthesise their secondary metabolic products during the dormant phase of their growth cycle. According to one theory, filamentous *actinomycetes* were observed to occur regularly in mycelium spores or small fragments. Germination happens when the conditions for growth are optimal, and it shows itself as the appearance of filaments with branches or rods that eventually mature into single-celled mycelia. In most cases, the resulting hyphae are aseptate. The vegetative mycelia develop within the substrate of the solid media, while the aerial mycelia emerge from the vegetative development. *Actinomycetes* are predominantly propagated through specialised sporulation bodies, as observed in Streptomycetes. Alternatively, propagation occurs through the hyphal terminal end of the developed mycelial network in non-sporulating genera such as Nocardia, Mycobacterium, Rhodococcus, Gordonia and Dietzia.

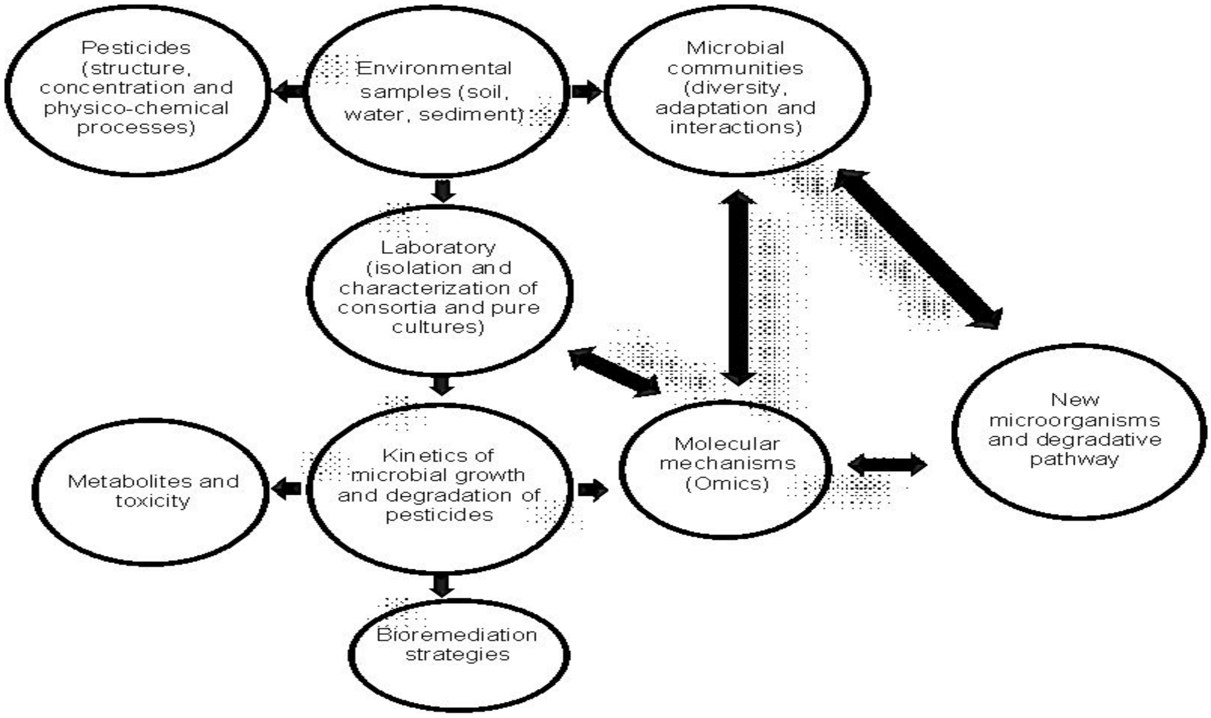

Image 1: This illustrates the connections between pesticides, microbial communities, and the revelation of new biodegradation processes. "Omics" refers to the high-throughput-based characterisation of biomolecules, a fundamental aspect of bioprocesses. This includes genomics (DNA), transcriptomics (mRNA), proteomics (protein), and metabolomics (metabolites).

2.4 Isolation of *Actinomycetes*

As empirical evidence demonstrates, application utilising unconventional selection methods paves the way for uncovering natural bioactive chemicals of previously unidentified and significant importance. Because they have characteristics that make them develop more slowly than other soil bacteria, *actinomycetes* can be difficult to isolate from mixed microflora found in nature. Nevertheless, several technologically effective solutions are available [86]. The selection of substrate is a crucial factor to consider. The retrieval of *actinomycetes* from various sources, such as soil, freshwater, and marine environments, has been documented in academic literature. There should be discernible distinctions among the organisms attainable in marine and terrestrial habitats. During the screening process of *actinomycetes* extracted from the shallow sea region, it was observed that certain antagonistic *actinomycetes*, specifically those that produce vancomycin, were more frequently isolated than those found in terrestrial soil. A limited quantity of *actinomycetes* was discovered to exhibit tardiness and generate novel antibiotics or bioactive substances through specifically designed circumstances. Isolating *actinomycetes* from marine environments provides a basis for exploring novel antibiotics and *actinomycetes* discovery [87].

2.5 Selective Media for Growth of *Actinomycetes*

Chemical agents with antifungal and antibacterial properties, such as sodium propionate and phenol, have been incorporated into the isolation media to inhibit or slow down the proliferation of moulds and bacteria, thereby promoting the growth of *actinomycetes*. However, implementing these modifications within permissible concentrations often facilitates impurities accumulation and may impede actinomycetes' growth at elevated thresholds. Utilising mineral salts in chitin agar has been found to enhance the efficacy of actinomycete isolation from water compared to chitin agar without mineral salts. According to a study [88], chitin agar demonstrated more excellent selectivity than other media in isolating *actinomycetes* from soil and water samples.

2.6 Incubation of *Actinomycetes*

Actinomycetes species that produce antibiotics grow best at temperatures between 25 to 30^0 C. *Thermophilic* microorganisms are cultured between $40\text{-}45^0$ C. In contrast, psychrophilic microorganisms are cultured at 4 to 10^0 C temperatures. The recommended incubation period for plate isolation ranges from 7- 14 days. More extended incubation periods have frequently been overlooked due to the sluggish growth of *actinomycetes*, which are unsuitable for commercial and industrial fermentation. Nevertheless, the initial proliferation of certain bacterial species can alter the isolation plate's nutrient environment by providing growth factors. To isolate new actinomycete species, it may be necessary to extend the incubation time by a month [89].

2.7 Selecting Colonies

For the isolation procedure, selecting an appropriate colony is a significant time-intensive process in biotechnology. The screening plan's objectives will determine the approach, and colonies could overlap significantly in the screening process. To isolate microorganisms, it is necessary to apply additional rational methods. Researchers typically use a stereomicroscope to select candidate colonies and transfer growth using a pointed piece of wood. Using suitable techniques, it is possible to choose and identify small colonies. The coarse timber tips contain sufficient spores or hyphal fragments to transfer successfully. The selection of the proper site for sample collection, the enrichment technique's goal, an understanding of the isolate's secondary metabolite, and the purpose of formulating culture media are crucial factors in identifying novel and promising isolate strains.

2.8 Identification of *Actinomycetes*

Several *actinomycetes* species can be observed on standard bacterial media utilised in the laboratory, including brain heart infusion agar, nutrient agar, trypticase agar, blood agar, and starch casein agar. Spore actinomycetes need specific media to facilitate segregation and increase the formation of pigment and the distinctive shape of spores. Several of these media are not commercially accessible and require laboratory preparation using colloidal chitin, plant material decoctions, and soil extract. When Streptomyces species on nutrient agar are sub-cultured in a better growth medium, like oatmeal or inorganic salts starch agar, Upon growing on nutrient agar, the Streptomyces species can produce a pale yellow colony with aerial mycelium that is chalky white and spirals of arthrospores. This contrasts the pale, complex colonies and shiny appearance observed on nutrient agar. The hyphae, which emerge from spores or mycelium fragments, extend into the agar and form substrate mycelium. The hyphae then proliferate and aggregate on the agar's top layer, forming a durable and resilient colony. The density of the colony and its ability to maintain a regular pattern is directly influenced by the chemical mixture or composition of the medium. *Actinomycetes*-derived Nocardio has fragmentation, with hyphae disintegrating into cocci and rods, resulting in the formation of territories that are either flexible or fragile [90]. The classification and characterisation of *actinomycetes* involve a polyphasic taxonomic approach that includes phenotype and phylogeny methods [91]. Phenotypic techniques involve morphological, biochemical, and physiological characterisation, whereas phylogenetic report utilises molecular methods. Based on our fundamental observation, it is imperative to focus on growing cultures on diverse media; a few examples of appropriate media are inorganic salts-starch agar (ISP4), oatmeal agar (ISP3), extract-malt extract agar (ISP2), and glycerol-asparagine agar (ISP5). During the final stages of culture maturation, the growth and morphologies of several different strains of Streptomyces are observed, resulting in the formation of significant spore mass. This is done so that the substrate mycelium's colour, the aerial spores' mass, and the diffusible pigment's production can all be determined. When working with different isolates, colour grouping is effective in differentiating them. This cluster of *actinomycetes* was classified together due to their similar morphological and physiological characteristics, particularly colour. The morphology of the sporophores is the subject of morphological observation because it is a characteristic of *actinomycetes* that has been thoroughly considered, is stable, and is clearly defined. On the other hand, this will only work if there is no risk of strain degeneration, which could be caused by incorrect maintenance or culturing. The aerial mycelium is a characteristic feature observed in specific genera like Streptomyces. The colony is enveloped by a hydrophobic cover and free, straight hyphae that extend into the air. The hyphae undergo pigmentation changes during spore formation, resulting in various colours from their original white appearance. The colonies are velvety or powdery, distinguishing them easily from typical bacterial colonies. The mycelial structure of Streptomyces species is characterised by the absence of spore chains commonly observed in other fungi that grow on a substrate. These spores are known as arthrospores and are specific segments of hyphae. Arthrospores are a distinct category of spores generated by select strains of bacteria and fungi. Arthrospores are distinct from conventional spores in that they arise from the disintegration of hyphae or filaments rather than from the emergence of specialised reproductive organs. The production of arthrospores by Streptomyces bacteria is not a documented phenomenon. Instead of conventional spores, the bacteria generate alternative spore variants, aerial or substrate spores. These spores are engendered through the maturation of specialised reproductive structures located on the apices of aerial hyphae or within the substrate where the bacteria are cultivated. Streptomyces aerial spores are typically produced at the tips of aerial hyphae and are commonly organised in a chain-like configuration. The spores are disseminated through various environmental factors, predominantly wind, and serve as a significant mechanism for bacterial propagation to novel sites. In contrast, spores of the substrate variety are generated within the substrate where the bacteria are cultivating, and their role in dissemination is typically negligible. The spore wall of an arthrospore is significantly thicker and enclosed in a hydrophobic sheath that may or may not contain hairs or spin. Identifying even the most enigmatic *actinomycetes* can be significantly aided by adhering to the basic principles of isolating colonies grown aerobically on egg-containing media or blood agar and conducting biochemical tests using molecular techniques and advanced taxonomies.

2.9 Optimizing Production of Bioactive Compounds from *Actinomycetes*

When looking for new and innovative pharmaceuticals, increasing the number of bioactive compounds produced by *actinomycetes* is of the utmost importance, particularly those with antibiotic properties. Among the most critical tactics are the selection and enhancement of strains, the optimisation of culture conditions, the investigation of co-cultivation and microbial interactions, the elicitation and metabolic engineering, and the refinement of downstream processing and product recovery. These methods can maximise compound yields while also contributing to the creation of new medications and lucrative bioproducts. Scientists have used various physicochemical, molecular, nutritional, immobilisation, and controlled mutational procedures to make *actinomycetes* species generate more of any bioactive substance. The regulation and management of physical-chemical and healthy variations is a fundamental and established technique for maximising production.

Furthermore, genetic engineering, mutagenesis, and immobilisation methodologies are efficacious means to augment production. Recent advancements in software and bioprocessing tools have led to the development of several software-based techniques that can be used to optimise the production of end products in bioprocessing and fermentation. Utilising an appropriate statistical plan, Response Surface Methodology (RSM) is a contemporary and significant technique for achieving this objective [92]. The ability to cultivate on nutrient agar, Muller-Hinton agar, and trypticase soy agar enriched with calcium chloride is exhibited by many actinomycete species, particularly those belonging to the genus

Streptomycetes. These species can synthesise targeted bioactive compounds in addition to all carbon and nitrogen sources. The development of bioactive chemicals from specific isolated *actinomycetes* species is also influenced by factors such as oxygen, temperature, pH, ions, etc [93]. The media's composition, particularly regarding glucose and phosphate, recognised suppressors for creating specific metabolites process, significantly affects antibiotic and bioactive chemical production. New biologically active secondary metabolites can only be successfully produced under controlled laboratory conditions. The study aimed to investigate the impact of growth conditions on the bioactivities of a diverse range of *actinomycetes* and to explore the potential of chemical or growth supplements in stimulating antimicrobial production. The productivity of the collected species was evaluated under varying growth conditions and with the addition of different additives. Results indicated that starch (1% w/v), Peptone (0.8% w/v), and a pH of 10 were the most effective conditions in activating the production of antibiotics among the 40 states assessed. There is no problem acquiring any criteria, and the costs are minimal.

Additionally, there is a minimal variance from one batch to the next [94, 95]. Some strategies are used to get a more significant yield amount or higher yield value than before. This leads to a higher level of production. An effective fermented design, optimisation of process parameters, optimisation of the medium, and recombination in microorganisms can all increase the yield [96]. In addition, the strain that was utilised for this research was improved through various techniques, such as immobilisation and mutation [97].

2.10 The Significance and Divergence of *Actinomycete*-Formed Secondary Metabolites.

Secondary metabolites are organic molecules that may support the microorganism's proper growth, reproduction, and development. These metabolites are produced by actinomycetes, which are a type of bacterium. The filamentous structure of actinomycetes distinguishes them from other types of gram-positive bacteria. They are well-known for their capacity to produce a wide variety of secondary metabolites, each exhibiting a unique set of biological properties, and this ability has earned them widespread recognition—antibiotics, fungicides, viricides, cancer treatments, and immunosuppressants all attribute secondary metabolites. Uses for secondary metabolites produced by microorganisms range from antibacterial agents and enzyme technology to pigment manufacturing and antitumor agents against cancer cells. The lexicon of natural products from *actinomycetes* includes information on over 7,000 substances. *Actinomycetes* have garnered significant attention in industrial manipulation due to their capacity to produce important secondary bioactive metabolites. *Actinomycetes* have been observed to produce a substantial amount of antibiotics, which make up roughly 70% of the natural antibiotics utilised in clinical medicine. *Actinomycetes*-derived antibiotics, including streptomycin, erythromycin, tetracycline, and vancomycin, have significantly transformed the management of bacterial infections. *Actinomycetes* can produce antifungal agents that can effectively combat systemic and superficial fungal infections in addition to antibiotics. Polyene macrolide antibiotics, such as amphotericin B and nystatin, are among the agents that fall under this category. The *Streptomyces* genus is the most extensive group of organisms that produce antimicrobial agents. It contributes up to 80% of the actinomycetes from natural sources [98]. Despite numerous antimicrobial compounds, these are believed to represent only a minor portion of the bioactive compounds that members of the Streptomyces genus can produce. Identifying novel secondary metabolites from typical *actinomycetes* has become increasingly challenging due to the prevalence of costly rediscoveries of known bioactive compounds during screening efforts. Various approaches have addressed this issue, including the meticulous isolation and screening of relatively rare *actinomycetes* [99]. *Actinomycetes* have exhibited promising potential in environmental applications, including bioremediation, biocontrol, and bioprospecting, through the production of secondary metabolites. Some strains of *actinomycetes* can make enzymes that are good at breaking down contaminants like hydrocarbons and polychlorinated biphenyls (PCBs). This attribute of *actinomycetes* can aid in the remediation of contaminated environments. The diverse range of secondary metabolites synthesised by *actinomycetes* highlights their importance and potential in drug discovery and biotechnology.

The discovery of gentamicin, an aminoglycoside antibiotic derived from Micromonospora echinospora and Micromonospora purpurea, has sparked a renewed interest in the exploration of non-streptomycete genera within the *actinomycetes* group to discover new antibiotics. The screening procedures yielded positive results, and a diverse range of antimicrobial compounds was extracted from non-streptomycete species. Vancomycin-type glycopeptides were found to be produced by various species of Amycolatopsis and Actinomadura. Actinomadura species have been found to contain naphthacene-quinone and macrolactam antimicrobial compounds. Similarly, Saccharopolyspora and Micromonospora strains have been discovered to produce various macrolide-type antibiotics [100]. *Actinomycetes* are known to produce multiple compounds of significant value, such as immunomodulators, vitamins, and enzymes that are utilised as biocatalysts in diverse industrial applications, in addition to antimicrobial compounds [101]. According to the research findings, it has been reported that Streptomyces species can produce over 2,400 distinct biologically bioactive secondary metabolites.

2.11 The application of secondary metabolite products derived from *Actinomycetes*.

Actinomycetes' inherent metabolic diversity and extensive interaction history with the surrounding environment are the primary reasons for focusing on these organisms in biotechnological applications. *Actinomycetes* are a microorganism that lacks a nucleus and exhibits various characteristics, including morphology, biochemistry, culture, and physiology. These microorganisms can potentially make many biologically active substances, such as antimicrobial agents, immunomodulators, enzyme inhibitors, enzymes, and growth-promoting compounds that animals and plants can use [102]. *Actinomycetes* are a type of bacteria that is characterised by their filamentous structure. They have become known for their capacity to synthesise a vast array of secondary metabolites, which have been found to have a variety of applications in various fields. The products offered by the company encompass a range of therapeutic categories such as antibiotics (streptomycin, tetracycline), anticancer agents (doxorubicin, daunorubicin), antifungal agents (amphotericin B, nystatin), immunosuppressive agents (rapamycin, tacrolimus), enzymes (cellulases, xylanases), biocontrol agents, bioremediation agents, and biosurfactants. The compounds above are applied in various industries, such as pharmaceuticals, agriculture, and biotechnology. Streptomyces is widely acknowledged as a prominent member of the antimicrobial-derived antibiotics within the *actinomycetes* species [103].

2.12 Antitumor compounds derived from *Actinomycetes*.

Chemotherapy is a prominent therapeutic approach employed in the fight against cancer. Many antitumor agents are derived from natural sources, either entirely or as derivatives. Microorganisms, particularly *actinomycetes*, are prolific producers of numerous natural compounds with diverse biological and bioactive properties, including antitumor activity [104]. *Actinomycetes* have garnered significant attention in cancer treatment due to their varied action mechanisms. These microorganisms have yielded antitumor compounds that have proven effective in combating cancer. The compounds above exhibit selectivity towards multiple aspects of cancer biology, including cellular proliferation, programmed cell death, formation of new blood vessels, and the spread of cancer cells to other body parts. Several widely recognised instances comprise anthracyclines, bleomycin, mitomycins, actinomycin D, and trabectedin. Current research endeavours aim to identify innovative bioactive agents that exhibit enhanced potency, selectivity and diminished adverse effects. In this context, antitumor compounds derived from *Actinomycetes* are considered potential contenders in the battle against cancer. The antitumor compounds that have been identified belong to a variety of structurally distinct categories, including anthracyclines, macrolides, enediynes, isoprenoids, indolocarbazoles and non-ribosomal peptides. These compounds have been found to induce apoptosis through various mechanisms, thereby exhibiting antitumor activity—the cleavage of DNA results from the inhibition of either topoisomerase I or II. Angiogenesis caused by a tumour can be stopped by blocking the action of critical enzymes involved in signal transduction, like proteases, mitochondrial permeabilisation, or cellular metabolism. Further, specific antineoplastic agents were extracted from strains of Streptomyces through intercalation with DNA duplex, resulting in adverse impacts on rapidly dividing cells by impeding the actions of DNA-dependent RNA polymerase.

2.13 The Role of *Actinomycetes* in Conservation and Ecosystem Maintenance

Actinomycetes play an essential role in ecosystem and environmental health because of their diverse metabolic capabilities. The potential uses of these microorganisms encompass a range of applications such as bioremediation, composting, fixing nitrogen, plant growth promotion, biocontrol, bioprospecting, and carbon cycling. Organisms with multiple functions are essential for maintaining healthy ecosystems and overcoming environmental challenges. *Actinomycetes* play diverse roles in the ecosystem. *Streptomycetes* are a type of saprophytic bacteria that break down organic matter. They are especially good at breaking down complex polymers like chitin, hemicelluloses, keratin, natural rubber, pectin, lignocelluloses, starch, and other synthetic compounds that can quickly get into the soil. *Actinobacteria* play a crucial role in the rhizosphere interaction by regulating plant growth and preventing the spread of disease-causing fungi to plant roots. The potential of *actinomycetes* as a form of biological control for soilborne root diseases in cultivated plants has been extensively studied, primarily through greenhouse experiments.

Moreover, various species of Streptomyces (along with select genera of *actinomycetes*) have been discovered to protect diverse plant species against fungal diseases transmitted through the soil. Several genera have been demonstrated to synthesise bioactive compounds that exhibit insecticidal and herbicidal properties. Similarly, some *actinomycetes*, like the genus Frankia, can also fix soil nitrogen. The microorganisms in question exhibit a broad range of host specificity and have been documented to establish mutualistic associations with over 200 species of angiosperms through root nodule symbioses.

2.14 *Actinomycetes*: Promising Agents for Bioremediation and Biodegradation

Actinomycetes possess diverse metabolic abilities that render them promising contenders for employment in bioremediation and biodegradation endeavours. The ability to secrete enzymes into the environment allows them to break down various organic compounds, including insecticides, petroleum hydrocarbons, and polycyclic aromatic hydrocarbons (PAHs). Furthermore, *actinomycetes* can engage in metal bioremediation via biosorption, biotransformation, and bioaccumulation, which enable them to sequester, immobilise, or convert heavy metals and metalloids into comparatively less harmful forms. The significance of microorganisms in environmental remediation is underscored by their ability to decompose synthetic polymers such as plastics and xenobiotics, which encompass pharmaceuticals and industrial chemicals. In addition, certain strains of *actinomycetes* play a role in augmenting phytoremediation by promoting plant growth. Research into the metabolic pathways, enzyme systems, and genetic regulation of *actinomycetes* is necessary to fully utilise them for bioremediation and biodegradation applications.

Additionally, biotechnological approaches such as genetic engineering and synthetic biology should be investigated to increase their efficiency further. The research shows *actinomycetes* are an effective decolourising agent for water-soluble synthetic reactive colours found in industrial effluents. The study demonstrated the decolourisation ability of *actinomycetes*, which reduced effluent colouration from 17% to 73%. This was achieved through either decolourisation or adsorption of the dyes onto the cells. The report documented the inclusion of various dyes such as anthraquinone, azo dyes, azo-copper complex, formazan-copper complex and phthalocyanine dye. Soil- and substrate-derived *actinomycetes* exhibit essential biodegradative capabilities. These microbes can metabolise potent molecules because they produce various extracellular enzymes. Composting relies heavily on the prolific activity of *actinomycetes*. Still, these microorganisms have evolved a wide range of techniques. These strategies range from short periods of rapid growth and sporulation to extended periods of slow growth and scavenging to stabilise their populations. It has been observed that *actinomycetes* are essential in the breakdown of pesticides. This process involves a wide variety of chemical structures, including sulfonylureas, organochlorines, s-triazines, organophosphonates, organophosphates, carbamates, acetanilides, organophosphonates, and triazinones. The herbicide degradation within the soil was reported to be carried out by *actinomycetes* that are indigenous to the ground. The essential *actinomycetes* species is Streptomyces, accompanied by Micromonospora, Nocardia, Actinoplanes, Actinomadura, and Dactylosporangium, later in the list of prevalent *actinomycetes.* Gordonia species have also been found to possess the unique ability to degrade rubber, as evidenced by their presence in contaminated water within the tire of a deteriorated automobile.

2.15 Actinomycete-Derived Enzymes: A Potential Resource

Enzymes are naturally occurring. Various microorganisms survive by hiding extracellular enzymes that can degrade many polymer forms. The ability of *actinomycetes* to produce a wide variety of secondary metabolites has earned them widespread recognition, including but not limited to antibiotics, immunosuppressants, and anticancer agents. In addition to the bioactive compounds, *actinomycetes* can produce various extracellular enzymes, such as proteases, cellulases, xylanases, amylases, and ligninases. The enzymes perform crucial functions in breaking down complex organic compounds, rendering them viable options for various industrial and biotechnological applications. *Actinomycetes* are microorganisms that produce many enzymes with potential or actual applications in biotechnology, therapeutic use, and clinical chemistry [105]. The enzymes derived from *actinomycetes* exhibit various potential applications that extend to multiple industries. Enzymes have been found to have potential benefits in agriculture, specifically in enhancing soil fertility and promoting plant health. This is achieved through the degradation of crop residues, which facilitates nutrient recycling and ultimately leads to improved plant growth. Enzymes from the genus *Actinomycetes* have been identified as a possible bioremediation solution for the degradation of environmental pollutants like pesticides, PAHs (polycyclic aromatic hydrocarbons), and heavy metals in polluted areas. Enzymes derived from *actinomycetes* are helpful in waste management by facilitating the degradation of lignocellulosic biomass. The process is essential in the manufacturing of biofuels and other valuable products. *Actinomycetes*-derived enzymes such as proteases, amylases, and others exhibit promising prospects for synthesising drugs, food supplements, and nutraceuticals in the pharmaceutical and food sectors.

Many biotechnological and industrial bioprocesses rely on enzymes, and *actinomycetes* constitute a significant source of these enzymes. Thus, very active proteases are used as additives to detergents or in the tanning industry, and all types of glycosidases are exploited to remove plant biomass. Fructose-rich syrups have been produced with success using glucose isomerases. In addition, mucolytic enzymes and bacteriolytic isolated from *actinomycetes* are purified and can be utilised to clarify alcoholic beverages such as beer and wine or as non-toxic food preservatives. Additionally, immobilised preparations of enzyme actinomycetes produce great potential for use in routine clinical diagnostic tests. After the production of antibiotics, the most significant yield that can be obtained from *actinomycetes* is enzymes. Enzymes derived from microorganisms find widespread application in various industries, including those dealing with food processing, the production of detergents, the pharmaceutical industry, the textile industry, medical treatment, molecular biology, and bioorganic chemistry. For a long time, *actinomycetes* were most famous as a source of antibiotics. They have also been recently identified as a possible source of numerous crucial enzymes.

2.16 Background of Agriculture in India

Agriculture contributes immensely to the Indian Growth Development Product (GDP), and a large population gets employment in the agriculture sector in India, which is also responsible for the growth and enhancement of the standard of life of millions. Most industries for raw materials depend upon agriculture, for example, the sugar industry, fruits and vegetable processing industry, cereals, legumes and oil seeds processing industry, beverage industry, cottage industry, etc. India is an agricultural country, and the utilisation of pesticides has enormously increased in our farming activities. Pesticides are chemical complexes that are responsible for the elimination of pests. Pests could be bacteria, fungi, worms, nematodes or insects accountable for the deterioration of the crops existing in the farms and the declining quality of soil texture. Therefore, pesticides are widely used to control or prevent plant diseases and pests. Pesticides are essential for promoting crop productivity, but the surplus amount of chemicals leads to ecological contamination, bacterial imbalance and health issues. Therefore, a typical pesticide should be bio-degradable as quickly as possible, and its end products must be non-toxic [106].

2.17 Role of pesticides in agriculture

In India, Pesticides are crucial for production in agriculture. The agricultural sector contributes about 22% of our country’s GDP and fulfils 70% of the nation’s livelihood. Today, in a global scenario, some of the top five agrochemical companies worldwide have captured 60% of the market share. As per a study conducted in 2006, the quantity of pesticides used was in agriculture production, accounting for around 67% of the total consumption [107]. Preferably, a pesticide must have a fatal impact on the target pests, but it should not be lethal for non-target species, specifically humans. However, pesticides pose risks to various ecosystems because of improper management. This endangers workers' health and the environment [108].

Further, pesticide residues that remain in soil taken up by the crops can directly impact human health by consuming food containing pesticide residues. A report by The World Health Organization suggested a million cases of pesticidal poisoning worldwide, providing sufficient data that pesticide affects different domains of human health pathologically [109]. The escalation of pesticide pollution leads to the emergence of communities known as 'cancer villages.' The term "cancer village" refers to a geographic area where the mortality rate from cancer is notably higher than the average mortality rate. This is primarily attributed to the pervasive contamination of pesticides, particularly in water sources [110]. The order of pesticide toxicity, based on their chemical composition, is as follows: organophosphorus (OP) pesticides are the most toxic, followed by carbamate (CB) pesticides, and then organochlorine (OC) pesticides. These primary intricate chemical compounds induce nervous system disorders [111]. The artificial organic compound has created so much pollution in the environment; therefore, there is an urgent need to come up with immediate solutions to save our environment. A recent study found that India still uses 104 pesticides that have been outlawed in other nations. Only 66 of these pesticides have been considered for review by the Indian government. The Ministry of Agriculture, Govt. of India, on 8 August 2018, put out a notification banning 18 neonicotinoid pesticides in the country. Twelve pesticides have been outlawed immediately, and another six will be eliminated gradually [112].

2.18 Monocrotophos pesticides and their impact on human health

Out of the various pesticides used in agriculture, The exceedingly dangerous organophosphate insecticide known as monocrotophos is utilised extensively and freely accessible in India. Produce as diverse as castor, olive, maise, rice, citrus fruits, sorghum, soybeans, sugar cane, peanuts, sugar beet, potatoes, onion, drinks, spices, and tobacco all benefit from this pesticide's widespread application. Soil stress and fertility loss are maximised when these herbicides are used excessively. The accumulation of such pesticides also imbalances the water level in the water table, and the natural degradation of the same is not easy. Monocrotophos (MCP) is a non-specific systemic organophosphorus (OP) pesticide and acaricide. It is used to combat common pests such as Helicoverpa sp., budworm, stem borer, aphids, caterpillars, mites, jassids, scales, and locusts, among other things. Monocrotophos (MCP) is a non-specific systemic organophosphorus (OP) pesticide and acaricide. It is used to combat common pests such as Helicoverpa sp., budworm, stem borer, aphids, caterpillars, mites, jassids, scales, and locusts, among other things. It is manufactured from mono-chloro-monomethyl acetoacetamide and trimethyl phosphate, and its water-solubility allows it to penetrate plant tissue rapidly. It has widespread application in agriculture, particularly in producing cotton, sugarcane, groundnut, maize, rice, soybean, vegetable, and ornamental crops. It has general application in agriculture, particularly in growing cotton, sugarcane, groundnut, maize, rice, soybean, vegetable, and ornamental crops. Only bioremediation is a perfect solution, which takes 12-16 days duration for bio-degradation [113]. According to the World Health Organization's report from 2009, accidental poisonings are primarily caused by monocrotophos pesticides. The utilisation of monocrotophos pesticides in various states was documented in a report from 2001 to 2006. The findings indicated that Andhra Pradesh had the highest usage rate, with a recorded amount of 2,779 metric tonnes. Punjab followed with 1,274 metric tonnes, Gujrat with 865 metric tonnes, Kerala with 169 metric tonnes, Bihar with 823 metric tonnes, West Bengal with 169 metric tonnes, Madhya Pradesh with 597 metric tonnes, and Tamil Nadu with 522 metric tonnes. Furthermore, Karnataka produced more than their respective states combined (103 megatons).

In 2000, a significant number of fatalities, totalling 1531, were attributed to the extensive use of pesticides. Among these cases, 609 were linked to the consumption of organophosphorus pesticides, while the highest number of insecticide poisonings, amounting to 86 patients, was reported due to the ingestion of monocrotophos.

Organophosphates like monocrotophos are found nationwide and are utilised a lot in agriculture. It is a direct-acting cholinesterase inhibitor that can get through the skin. The effects can be seen in minutes or a day, but the side effects are identical to those of other organophosphate compounds. It affects the nervous system because it stops cholinesterase from working. Muscle weakness, hazy vision, excessive sweating, confusion, vomiting, discomfort, and tiny pupils are symptoms of human poisoning. This could cause you to vomit, have diarrhoea, feel sick, get a headache, have stomach cramps, etc. When someone is severely poisoned by monocrotophos, they can go into cardiac arrest or stop breathing, leading to death[2]. FAO and WHO, two of the most significant health and agriculture groups, advised countries to list pesticides with hazardous ingredients. Many countries were involved, including Australia, China, the European Union, Cambodia, Laos, Indonesia, the Philippines, and Vietnam. Sri Lanka, Thailand, and the United States have all banned the use of monocrotophos. Urgent action must be taken to remove this from the market. Monocrotophos is also forbidden in many developing countries in Asia because it poses serious health risks. India is very aware of the risk of pesticides on human health. However, pesticides like monocrotophos are regularly made, utilised, and exported from India in rural India. It is cost-effective and crucial for agricultural output [114]. Image 1 depicts the chemical structure of the pesticide monocrotophos.

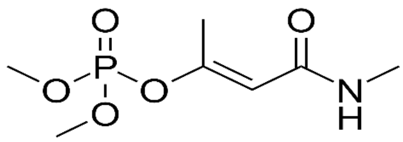

Image 1: Monocrotophos: its chemical structure ($C_7H_{14}NO_5P$)

2.19 Bioremediation: The most eco-friendly Approach for the degradation of xenobiotics

Different eco-friendly methods have been used in the last two decades to clean up the contaminated environment, For example, using diverse microbial species.

This approach is commonly referred to as bioremediation. Compared to conservative physicochemical methods, this is regarded as a preferable approach that is more effective in repairing soil functions [115]. Bioremediation is also utilised to convert hazardous substances into less harmful components. Microorganisms are crucial to the process of breaking down and mineralising these pollutants. Bioremediation is an essential sustainable technology for analysing and decreasing the pollution caused by human activities. Bioremediation makes use of particular bacteria called bioremediation to clean up polluted areas. Ex-situ bioremediation and in-situ bioremediation are the two categories typically used to describe the classification of bioremediation technology. In the process known as "in situ bioremediation," contaminated materials are cleaned up and removed directly from the site where they were polluted. On the other hand, ex-situ bioremediation refers to treating contaminated material in a setting that is remote from the original contamination site. Bioventing, bioleaching, biostimulation, land farming, composting, bioaugmentation, rhizofiltration, and phytoremediation are some of the bioremediation technologies that are currently available [116].

Both biodegradation and bioremediation utilise microorganisms for the modification or breakdown of pesticides. Therefore, they are practically the same process. The primary differentiation between biodegradation and bioremediation lies in their respective nature, with biodegradation being a natural process and bioremediation being a technological treatment. Soil pH, temperature, nutrient availability, water potential, and metabolite or pesticide concentration limit the growth of microorganisms capable of decomposing pesticides [117].

2.20 Actinobacteria: the most promising candidate which must be focused for bioremediation

It is now widely accepted that bioremediation of organic chemicals and heavy metals is practical; nevertheless, each procedure is often isolated. For the bioremediation of co-contaminated locations, a biological process with multiple functions is believed to be necessary. Recently, *actinomycetes* have gained prominence as potential bioremediation work targets. These microorganisms, which are cosmopolitan, each have their own set of traits that make them particularly useful in the breakdown and detoxification of various toxins. Recently, *actinomycetes* have gained prominence as potential bioremediation work targets. These microorganisms, which are cosmopolitan, each have their own set of traits that make them particularly useful in the breakdown and detoxification of various toxins. Looking closely at it, we find that the actinobacteria members of the phylum bacteria reflect significance as they have been demonstrated to possess potential as effective agents for the biological remediation of contaminants such as heavy metals and pesticides. Due to their robust metabolic capabilities, they can help degrade environmental pollutants like pesticides, herbicides, and medicines. Several studies are being made on the constituents of the phylum Actinobacteria due to their capability to bioremediate co-polluted soils [118]. In addition to thriving in extreme environments, actinobacteria are well-suited for bioremediation initiatives in highly polluted regions. These organisms survive and successfully perform their biodegradation tasks because they contain stress-response systems that allow them to adapt to severe pH, temperature, and osmotic circumstances. It is established that Actinobacteria is a diversified phylum having six significant classes, 19 orders, 50 families and 221 genera. The actinobacteria are Gram-positive, high G+C-rich bacteria [119].

The member of the phyla, *Streptomyces sp* represents a valuable reservoir of naturally occurring compounds that can secrete enzymes and secondary metabolites that behave as antimicrobial and anti-tumour agents. These well-known organisms, *Actinomycetes,* are responsible for 45% of all the secondary metabolites, and *Streptomyces* genera are responsible for 80% of the total composition [120]. Single isolates of bacteria can mineralise certain xenobiotic pesticides. To achieve complete degradation, bacterial consortiums are typically necessary in most cases. Immunosuppressants, phytotoxins, biopesticides, nanoparticles, probiotics, enzyme inhibitors, and the various enzymes and proteins involved in the degradation of complex polymers and biomolecules are all examples of the types of molecules that fall into this category [121, 122]. It has been observed that the co-metabolism of pesticides is commonly found within the group of bacteria. Compared to the process of pesticide degradation carried out by Gram-negative bacteria, the amount of information currently available regarding the molecular pathways involved in the actinomycete biotransformation of pesticides needs to be increased. Most of this important class of soil bacteria progress has been highly hindered by appropriate molecular genetic tools. This limiting factor needs to be mastered so that *actinomycetes*' biodegradation and biotransformation capabilities can be used more effectively to use applications for bioremediation and to build transgenic herbicide-resistant crops [123]. Actinobacteria can establish symbiotic associations, augmenting phytoremediation endeavours, which is an additional advantage.

Rhizosphere bacteria play a critical part in the breakdown of organic pollutants, making it easier for plants to obtain vital nutrients, boost plant growth, and make them more resistant to the adverse effects of environmental stressors. *Actinobacteria* has immense biotechnological potential as it is capable of eradicating organic as well as inorganic pollutants. Due to this, it has gained much attention in recent years and thus is preferred ideally for bioremediation [124].

2.21 Review of studies done on biodegradation of pesticides

The studies were performed on two cultures viz. Using an enrichment and adaptation culture approach, *Bacillus megaterium* MCM B-423 and *Arthrobacter atrocyaneus* MCM B-425 were isolated from soil treated with MCP. The MCP degradation capacity of the isolates was determined to be 93% and 83%, respectively, when exposed to a synthetic medium that contained MCP at a concentration of 1000 mg/l. This degradation occurred within eight days under shake culture conditions at 30^0C. An unknown substance was utilised by different cultures to break down the Monocrotophos pesticide into ammonia, carbon dioxide, and phosphates [125]. Previous studies described the genera Actinobacteria. The members of these genera are found extensively in as well as terrestrial environments, resulting in cosmopolitan distributions. They provide critical ecological and environmental functions, including recycling materials, breaking down complex polymers, and producing bioactive compounds. The ability of actinobacteria to clean up both organic and inorganic contaminants in the environment illustrates their valuable biotechnological potential. The increased interest in Actinobacteria as potential options for bioremediation is directly related to the rise in significance of this method, a consequence of the widespread release of harmful substances into the environment. Despite their detrimental effects on the ecosystem's delicate balance gradually becoming clear, pesticides are among the most commonly utilised organic pollutants.

Similar to this, the widespread use of heavy metals in industrial operations has resulted in highly contaminated places all over the world. In the past 15 years, several researchers have focused on utilising actinobacteria for environmental cleanup. Actinobacteria's bioremediation capacities have been improved through bioaugmentation, biostimulation, cell immobilisation, biosurfactant synthesis, defined mixed culture design, and plant-microbe systems [126]. The biotransformation and biodegradation of pesticides by *actinomycetes* have significant promise. Evidence shows that members of this family of gram-positive bacteria can break down various types of pesticides, including s-triazines, acetanilides, carbamates, organophosphonates, triazinones, organochlorines, sulfonylureas, and organophosphonates. However, while a single isolate can metabolise some of these xenobiotic pesticides, this is only the case for some, whereas full breakdown frequently requires bacterial consortia.

This particular bacterial community usually exhibits pesticide metabolism. In contrast to the extensive knowledge of the molecular mechanisms underlying pesticide degradation by gram-negative bacteria, there is a need for more information regarding the bio-transformations of pesticides by *actinomycetes*. The absence of appropriate molecular genetics techniques for most soil bacteria in this influential group has significantly impeded advancements in this field. The resolution of this limitation would facilitate the more effective utilisation of the biodegradative and biotransformative capacities of *actinomycetes* in various domains, including but not limited to bioremediation and the development of genetically modified herbicide-resistant crops [127]. The bacteria exhibit natural functionality in their environment; however, specific modifications can be implemented to expedite the degradation of the pesticide by the microorganisms within a reduced timeframe. The capacity of microbes is occasionally employed as a technological solution for eliminating contaminants from their original location. A comprehensive understanding of the target microorganism's biochemistry, physiology and genetic information can enhance the efficacy of microbial processes for bioremediation, resulting in precise and error-free outcomes and consistent microbial performance. Identifying gene encoding for enzymes in various pesticides offers novel insights into the microbial potential for pesticide degradation and the development of a super strain to accelerate bioremediation efforts [22]. Several research investigations have assessed the genotoxic and cytotoxic impacts of monocrotophos, an organophosphate insecticide, on human lymphocytes cultured in vitro. According to the research findings, it was observed that monocrotophos exhibited a high level of toxicity towards lymphocyte culture. The utilisation of karyotyping facilitated the identification of chromosomal abnormalities induced by monocrotophos. The study results suggest that there was a statistically significant increase ($P < 0.05$) in the incidence of satellite associations, breaks, and gaps compared to the control group, indicating that these observations carry statistical significance [128].

Organophosphorus compounds are extensively utilised insecticides, constituting approximately 34% of global insecticide sales. Pesticide-related soil contamination can happen after pesticides have been applied in the field and then handled in bulk at the farmyard. This can contaminate surface and groundwater or result from an accidently released chemical. According to several investigations, various marine and terrestrial habitats may contain organophosphorus chemicals. Because of their significant mammalian toxicity, it is crucial to eliminate these chemicals from the environment. Decontaminating contaminated ecosystems and eliminating nerve agents can be accomplished effectively and affordably through bioremediation. In 1973, Flavobacterium sp., the first bacteria to break down organophosphorus chemicals, was discovered. Subsequently, several bacterial and some fungal species have been identified that can decompose a broad spectrum of organophosphorus compounds in both liquid cultures and soil environments [129].

Most bacteria appear to degrade organophosphorus compounds using the same biochemistry, with the first step of the process being catalysed by an enzyme known as organophosphate hydrolase or phosphotriesterase. The gene opd, which encodes for organophosphate hydrolase with the ability to degrade organophosphates, has been obtained from various taxonomically distinct and geographically diverse species. Following sequencing, this gene has undergone modifications to enhance its activity and stability and has subsequently been cloned in multiple organisms. Recently, genes exhibiting comparable functionality, though distinct sequences have been identified and analysed. The efficacy of engineered microorganisms in breaking down various organophosphorus pollutants, such as nerve agents, has been evaluated in studies [130]. Different researchers reported the bioremediation of monocrotophos pesticides (MCP). The isolates were obtained after growing on a 1% agar medium containing monocrotophos pesticide. These isolates were found to have MCP degradation capabilities and are found to be bacterial and fungal strains, respectively [25]. In one study, The specimens were obtained from the sediment accumulated at the base of a chemical plant's wastewater treatment facility. The cellular morphology, physiological, and chemotaxonomic characteristics were analysed. The strain was confirmed as *a Paracoccus* species. Under various growing conditions, the strain's capacity to mineralise monocrotophos was examined. The discovered strain was observed to break down further organophosphorus insecticides and amide herbicides. The report reveals that the initial breakdown of monocrotophos in M-1 is aided by a cytosolic protein that is constantly produced and is considered to be the critical enzyme(s) responsible for this process. The research indicated that when M-1 (10 CFU/g) was added to flavour-aquic soil and high-sand soil containing monocrotophos (50 mg/kg), the rate of degradation was significantly higher than in soil that did not receive any inoculation [131].