

ISOLATION, IDENTIFICATION AND CHARACTERISATION OF PESTICIDE (PROFENOFOS) DEGRADING BACTERIA FOR BIOREMEDIATION

PROJECT WORK

Submitted in partial fulfillment of the requirements for
the award of Bachelor of Science degree in Microbiology

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SATHYABAMA

**INSTITUTE OF SCIENCE AND
TECHNOLOGY (DEEMED TO BE
UNIVERSITY) CATEGORY -1**

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APRIL - 2024



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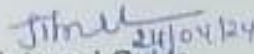
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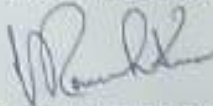
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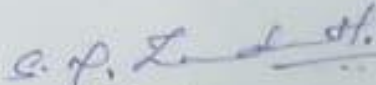
This is to certify that this Project Report is the bonafide work of ROHINTH.M (41300037) and SIVARAMAN.E (41300042) who have done the Project work entitled " ISOLATION, IDENTIFICATION AND CHARACTERISATION OF PESTICIDE (PROFENOFOS) DEGRADING BACTERIA FOR BIOREMEDIATION" under my guidance from January 2024 to April 2024.


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DECLARATION

We, Rohinth.M (41300037) and Sivaraman.E (41300042) hereby declare that the Project Report Entitled "ISOLATION, IDENTIFICATION AND CHARACTERISATION OF PESTICIDE (PROFENOFOS) DEGRADING BACTERIA FOR BIOREMEDIATION" done by us under the guidance of Dr.J.Theboral at the Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfillment of the requirements for the award of Bachelor of Science degree in Microbiology.

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ACKNOWLEDGEMENT

We were pleased to acknowledge my sincere thanks to The Board of Management of **Sathyabama Institute of Science and Technology** for their encouragement in doing this project and for completing it successfully. We are grateful to them.

We convey our thanks to **Dr. REKHA CHAKRAVARTHI**, Dean, **Science and Humanities** and **Dr. V. RAMESH KUMAR**, M. Phil, Ph.D, Head of the Department, Dept. of Biotechnology for providing us with the necessary support and details during the progressive reviews.

We would like to express our sincere and deep sense of gratitude to our Project Guide **Dr.J.THEBORAL** for her valuable guidance, suggestions and constant encouragement paved way for the successful completion of our project work.

We wish to express our thanks to all **Teaching and Non-teaching staff** members of the Department of Biotechnology who were helpful in many ways for the completion of the project.

ABSTRACT

In this research study, pesticide degrading bacteria were isolated from the agricultural soil samples collected from the Thirunelveli district. Initially, colonies were identified as pesticide-degrading by growing them at the constant concentration of 1.5 ul of profenofos. Then, the total of 12 efficient isolates (PS1- PS12) were tested for their efficiency by inoculating them on increased concentration of pesticide. Out of twelve isolates, two isolates (PS1, PS10) showed good growth even at 2.5 ul concentration of profenofos which were later characterized by several biochemical assays such as IMViC, phosphate solubilization test and Gram staining. PS1, one of the two isolates, showed good growth kinetics on liquid media supplemented with profenofos and was identified as Gram positive, bacilli shaped *Fredinandcohnia salidurans* and also degraded the pesticide profenofos with 70% efficiency which was confirmed by HPLC (High Proficiency Liquid Chromatography) analysis. Therefore, *F. salidurans* can be used in the bioremediation of pesticide contaminated soil.

Keywords: Bioremediation, pesticides, profenofos, HPLC, *Fredinandcohnia salidurans*

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LIST OF ABBREVIATIONS

BLAST	Basic Local Alignment Search Tool
DNA	Deoxyribo nucleic acid
FAO	Food and Agriculture Organisation
Fe/Ni	Iron/Nickle
HPLC	High Proficiency Liquid Chromatography
IPM	Integrated Pest Management
ITS	Internal Transcribed Spacer
MR-VP	Methyl red – Voges
Proskauer NA	Nutrient Agar
NCBI	National Center for Biotechnology Information
PCR	Polymerase Chain
Reaction pH	Potential of
Hydrogen	
UV	Ultra Violet rays
WHO	World Health Organisation
ZRC	Zymo Research Corporation

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CHAPTER 1

1. INTRODUCTION

1.1 PESTICIDES

Pesticides are chemicals or substances used to control, repel, or kill pests such as insects, weeds, fungi, and rodents that can harm crops, animals, or humans. They play a crucial role in protecting agricultural crops and controlling pests that can spread diseases or cause economic damage. Pesticides can be classified in various ways based on their chemical composition, mode of action, target organisms, and persistence in the environment.

1.2 CLASSIFICATION OF PESTICIDES

For the purpose of identification, pesticides have been classified into groups using various classification methods. However, there are mainly three popular methods used for pesticide classification such as, classification based on the “mode of entry, target pests, and chemical type” (Yadav and Devi, 2017). In addition to the above three methods, classifications based on toxicity level, and mode of action can also be found (Akashe *et al.*, 2018). In the classification based on ‘mode of entry’, the way how a particular pesticide come into contact with or enter into the target pest is considered. According to this classification, there are several classes of pesticides such as, “systemic, contact, fumigants, stomach poisons, and repellents” (Yadav and Devi, 2017; ..Akashe *et al.*, 2018). Based on the target pests, pesticides are grouped into several categories such as, “insecticides, fungicides, rodenticides, algaecides, nematocides, acaricides, larvicides, aphicides, molluscicides, miticides, ixodicides, bactericides and herbicides” (Gilden *et al.*, 2010; Yadav and Devi, 2017; Akashe *et al.*, 2018). Based on the type of chemical, pesticides can be again grouped into several classes such as, “organic, inorganic, synthetic, and biological” (Council_on_Scientific_Affairs, 1997). Moreover, the chemical composition

based classification of pesticides have grouped various pesticides into four main groups such as, “organophosphorus, organochlorine, carbamates, and

pyrethroids” (Yadav and Devi, 2017). In addition to that, “arsenic compounds, mercury compounds, copper compounds, bipyridylium compounds, coumarin derivatives, nitrophenol derivatives, phenoxyacetic acid derivatives, triazine derivatives, organotin, pyrazoles, and thiocarbamates” are also some groups of pesticides classified, based on chemical composition (WHO, 2010). Based on toxicity level, pesticides have further been classified into several groups such as, “extremely hazardous, highly hazardous, moderately hazardous, slightly hazardous, and unlikely to present acute hazard” (WHO, 2010; Akashe *et al.*, 2018). According to the mode of action, pesticides are classified into several groups such as, “physical poison, protoplasmic poison, respiratory poison, nerve poison, and chitin inhibition” (Akashe *et al.*, 2018). Of major classes of common pesticides used in the world, majority accounts for herbicides (47.5 %) followed by insecticides (29.5 %), fungicides (17.5 %), and other pesticides (5.5 %) such as, bactericides, acaricides, rodenticides etc. (De *et al.*, 2014; Sharma *et al.*, 2019).

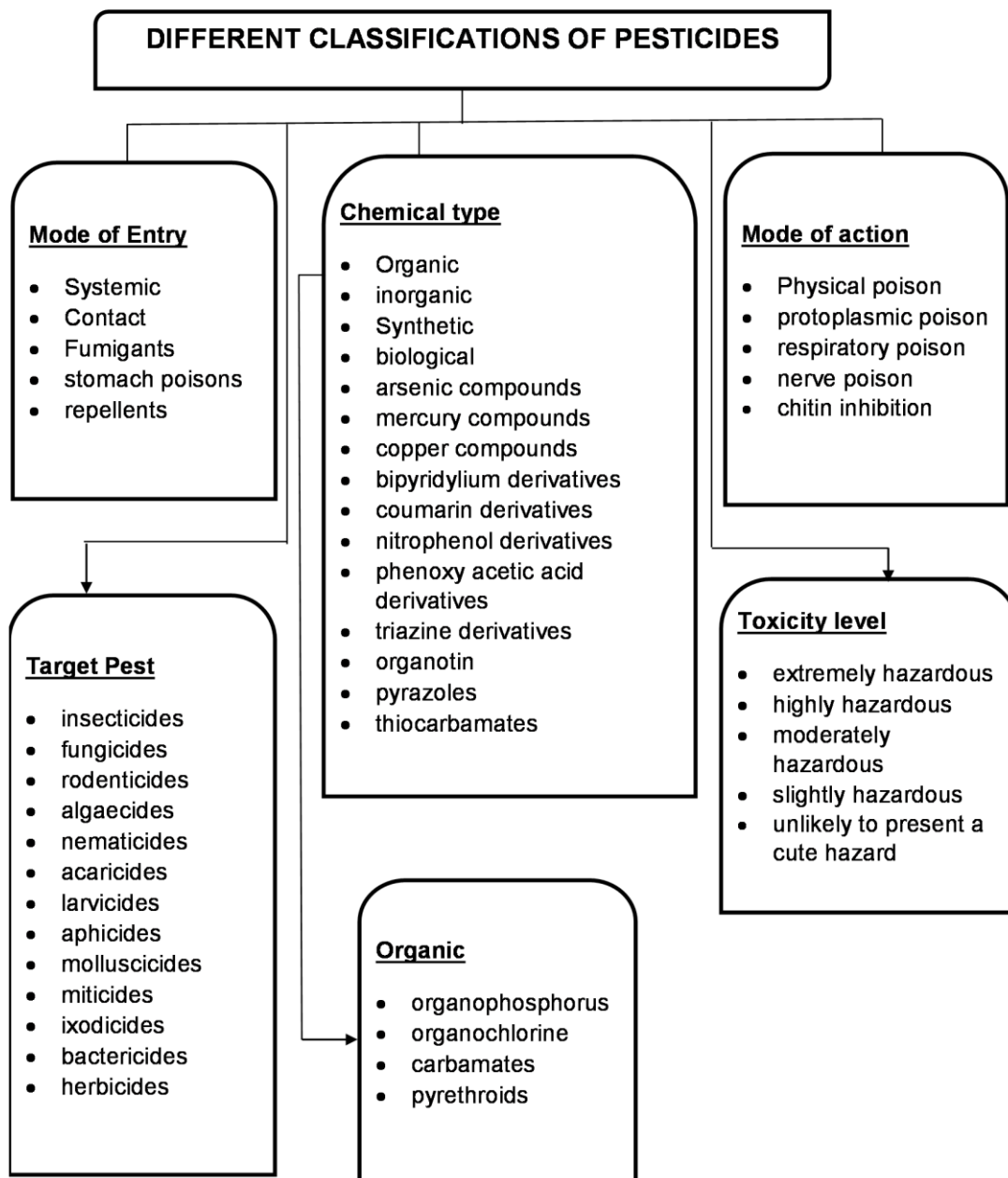


Fig 1.1: Classification of pesticides

1.3 WORLD WIDE USAGE OF PESTICIDES

The worldwide usage of pesticides has raised concerns due to their potential environmental and health impacts. The agricultural sector is the largest user of pesticides globally. Farmers use pesticides to protect crops from pests that can significantly reduce yields. In developing countries, where agriculture is

a major economic activity, pesticide use has been increasing to meet the growing demand for food production. There are different types of pesticides used worldwide, including insecticides, herbicides, fungicides, and rodenticides (Randika *et al.*, 2022). Each type targets specific pests or organisms that threaten crops or public health. Pesticide use has been increasing steadily worldwide over the past few decades. This trend is driven by factors such as population growth, changing food preferences, and the need to improve agricultural productivity. Governments around the world regulate the sale and use of pesticides to protect human health and the environment. Regulatory measures include setting safety standards, establishing usage guidelines, and conducting risk assessments for pesticides before they can be approved for use (Sharma *et al.*, 2019).

In response to the environmental and health concerns associated with pesticides, there is an increasing focus on promoting sustainable agriculture practices and alternatives to chemical pesticides. Integrated pest management (IPM) techniques aim to minimize pesticide use by combining biological, cultural, and mechanical methods of pest control. Pesticides are traded globally, with different countries having varying regulations and standards for their use. International organizations like the Food and Agriculture Organization (FAO) and World Health Organization (WHO) provide guidance on pesticide management and safety (De *et al.*, 2014).

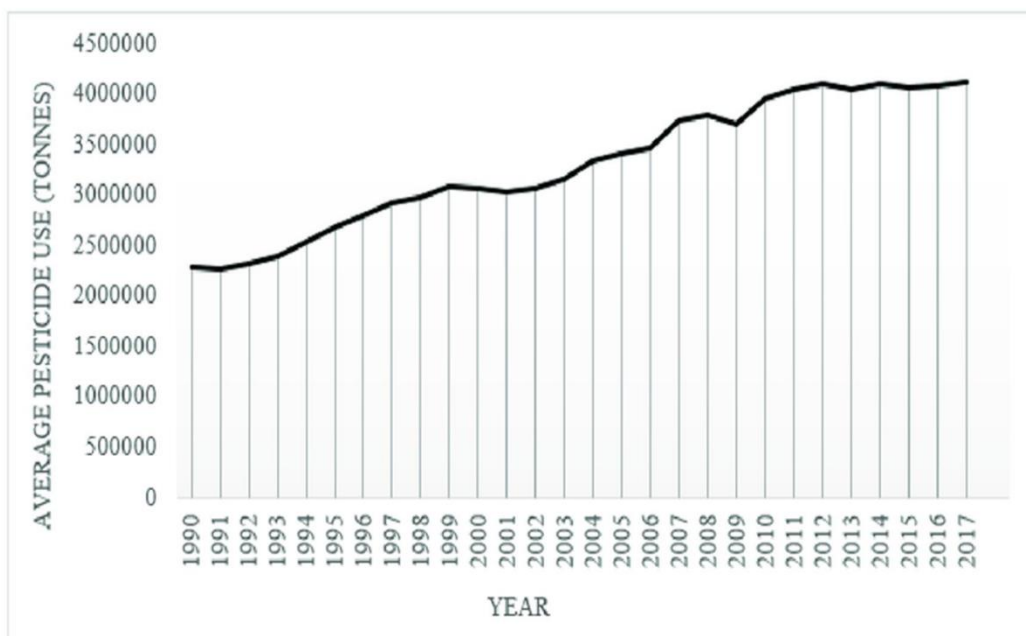


Fig 1.2: Increasing pattern of average world pesticide usage from 1990 – 2017 (Randika et al., 2022)

1.3.1 Pesticides as Environmental Pollutants

Although pesticides play a great role in agriculture, there is no doubt that they have some adverse impacts on the environment. Use of pesticide in high input agriculture is inevitable and the extensive usage has created a greater threat to the ecosystem as well as to the human health (Carvalho, 2017). Due to the high toxicity and high biological activity of pesticides, they hold an exclusive position out of the other environmental contaminants. The effect of pesticides is not only on the target pest but also on the other non-target organisms such as, earthworms, natural predators and pollinators. Not only the terrestrial animals but also most of the aquatic animals, planktons, birds and microorganisms are also affected by the toxic effect of pesticides (Ware, 1980; Flexner *et al.*, 1986; Smith and Stratton, 1986; Pereira *et al.*, 2009). This can directly affect the balance of the natural ecosystem. Most of the

previous studies have shown that only a very small percentage (0.3 %) of the entire amount of pesticides applied

to a land reaches the target pest whilst the rest goes somewhere else (Pimentel, 1995; van der Werf, 1996) and although the pesticide is applied into a small area, it can spread over a vast area. After getting released into the environment, pesticides may have many different fates such as, retaining in the air, absorbing into the soil or dissolving in the water etc. Pesticide residues in the soil can reach surface water bodies through surface runoff or may contaminate the ground water by percolation (van der Werf, 1996; Yadav and Devi, 2017). The toxic compounds in pesticides mixed with surface water bodies can directly affect aquatic organisms. Moreover, pesticide residues could be accumulated in the tissues of living beings and transferred from one level to the next through food chains (Chen and Mulchandani, 1998). Human beings can also be directly or indirectly affected by the toxicity of pesticides (Chen and Mulchandani, 1998; Curl *et al.*, 2002; Young *et al.*, 2005; Binukumar and Gill, 2011; Yadav and Devi, 2017).

1.3.2 Adverse effects of pesticide usage

Following the Second World War, there was a sharp rise in the manufacture and application of synthetic pesticides (Johnson and Talbot, 1983). The introduction of synthetic pesticides has greatly aided in the global control of pests in agriculture, resulting in an increase in crop productivity. However, the fate of these pesticidal compounds in the environment has remained unknown as they were applied to agricultural lands. Although the prolong existence of these chemicals may be an advantage for the sustained pest control, cumulative effect of residual accumulation can be adverse on the balance of environment and all living organisms including human beings. However, the nature of the byproducts of natural degradation is distrustful because the pesticide's toxicity could change as it transforms into another compound (Menzie, 1972; Gavrilescu, 2005). Furthermore, the permanence of pesticide residues remains unknown due to their diverse distribution across many environmental matrices (Darko *et al.*, 2008; Nag and Raikwar, 2011; Silva *et al.*, 2019; Heshmati *et al.*,

2020). In addition to having detrimental side effects on the environment, the extensive and careless use of pesticides has led to major health problems in humans and other living things (Jeyaratnam, 1985; Kumar *et al.*, 2013b; Sharma and Singhvi,

2017). Moreover, pesticide residue accumulation can happen in the tissues of living beings inhabited in pesticide contaminated environments and, transferred through food chains (Varo *et al.*, 2002; Akan *et al.*, 2013). On the other hand, Pesticide leaching into the soil and groundwater bodies is also an important cause to consider (Chen and Mulchandani, 1998). Although pesticides cause many adverse effects to the nature, necessity of pesticide usage is dramatically increased with the increase of world population. Increasing world population greatly increases the demand for food production leading to intensive agriculture, as there is limited land available for cultivation. This may also dramatically increase the future pesticide usage making the scenario worst (Stephenson *et al.*, 2001; Popp *et al.*, 2013). The adverse effects of pesticides are becoming increasingly clear on the natural ecosystem as well as on human health.

1.4 BIOREMEDIATION

In terms of overcoming these adverse impacts, discovery of rapid pesticide detoxification techniques is timely important. Bioremediation plays a great role in detoxifying residual pesticides in the environment. The capability of various microorganisms to degrade or detoxify pesticides has been exploited. In the process of bioremediation, harmful compounds of pesticides are broken down into harmless non-toxic compounds through microbial metabolism (Gavrilescu, 2005; Odukkathil and Vasudevan, 2013). Fungi, bacteria, cyanobacteria, actinobacteria and some of other microorganisms have been recognized with the capabilities of degrading pesticides compounds (Hutchinson *et al.*, 2001; Singh and Ward, 2004; Das and Dash, 2014). Since past, bioremediation has been used in waste water treatments. Now a days, this technology has been further developed and used globally (Iqbal and Bartakke, 2014). Moreover, bioremediation is considered as a safe, cheaper and environmentally sound method of sequestering harmful contaminants from the natural ecosystem (Das and Dash, 2014; Bhardwaj *et al.*, 2019).

Plant mediated bioremediation process is termed as phytoremediation (Singh and Ward, 2004). However, majority of identified soil microorganisms having such abilities are bacteria and fungi (Geetha and Fulekar, 2008;

Odukkathil and Vasudevan, 2013; Uqab *et al.*, 2016). Microbial bioremediation can effectively be used in detoxifying toxic pesticide residues built up in the environment and an ample number of studies have been conducted in this regard worldwide. In natural habitats, microorganisms are generally existing as consortia which are more efficient than single strains in degrading pesticides and as a result, a microbial consortium can degrade different types of pesticides simultaneously (Góngora-Echeverría *et al.*, 2020). In some studies, they have tested the bioremediation ability of some natural soil microorganism consortia whereas, some scientists have isolated microorganisms followed by testing the individual ability of microbes to degrade pesticides (McAllister *et al.*, 1996; Geetha and Fulekar, 2008; Massiha *et al.*, 2011; Werren, 2012). In addition to the soil and water inhabited bacteria, some studies have revealed that some microbes in the gut microbiota of some insects have the capability of degrading some insecticides (Almeida *et al.*, 2017).

1.4.1 Mechanism of Microbial Bioremediation

Microorganisms play a crucial role in the degradation of contaminants like pesticides, utilizing them as energy or nutrient sources. This degradation process results in the conversion of harmful substances into less or nontoxic byproducts, a principle underlying microbial bioremediation. Various studies have shown that microorganisms use pollutants as their sole carbon or nitrogen source, employing mechanisms such as aerobic, anaerobic, and chemolithotrophic metabolism, as well as enzymatic degradation (Bollag and Liu, 1990). Enzymes like oxidoreductases, monooxygenases, and hydrolases are significant in pesticide bioremediation, catalyzing reactions such as oxidation, hydroxylation, and hydrolysis based on the chemical structure of the pesticides. Co-metabolism, demobilization of contaminants, and the role of microbial genes in bioremediation have also been identified as important aspects in the process.

1.4.2 *Factors affecting* ***bioremediation***

Microbial bioremediation effectiveness is influenced by multiple factors, including the type of contaminant present, its concentration and distribution in the environment, the availability of suitable microbial populations, environmental

conditions (such as temperature, pH, oxygen levels), the presence of any inhibitors or contaminants that may interfere with microbial activity, and the nutritional requirements of the microbes (Das and Dash, 2014). Additionally, factors such as the interactions between different microbial species, competition for nutrients, and the ability of microbes to adapt and survive in harsh environmental conditions can all impact the success of bioremediation processes. The bioavailability of the contaminants to the microorganisms, as well as the availability of electron acceptors and donors, can significantly influence bioremediation efficiency. In addition, the design and implementation of the bioremediation strategy, such as the selection of appropriate microbial strains, inoculum size, and the method of delivery of microbes and amendments to the contaminated site, play crucial roles in determining the success of the remediation process (Boopathy, 2000).

1.4.3 *Prevalent Bioremediation Techniques*

Bioremediation has seen significant advancements, with a focus on gene editing, system biology tools, and recombinant DNA technology for pesticide bioremediation. Scientists are aiming to understand the genetics and biochemistry of biodegradation processes by natural microbes to develop a biodegradation network. Additionally, research is being conducted on using microbial glycoconjugates and microbial consortia for more effective bioremediation of pesticide contaminants. In terms of bioremediation strategies, both in situ and ex situ bioremediation techniques are being widely practiced, with methods such as bio-stimulation, bioaugmentation, biopiling, and composting showing promise in degrading pollutants in soil and water environments. The combination of different strategies has been proven to enhance the effectiveness of bioremediation, making it a viable approach for removing pollutants from the environment (Boopathy, 2000; Iwamoto and Nasu, 2001).

1.5 PROBLEM OF THE STUDY

The present study aimed to identify the bacterial isolate that able to degrade pesticide, particularly Profenofos, widely used pesticide in Tamil Nadu. Profenofos is an organophosphate pesticide widely used in agriculture to control a broad spectrum of pests, including insects, mites, and nematodes, on various crops such as cotton, fruits, vegetables, and cereals. Chemically, it belongs to the group of organophosphorus compounds, characterized by their mode of action targeting the nervous system of pests. Profenofos inhibits the activity of acetylcholinesterase, an enzyme crucial for the proper functioning of the nervous system, leading to the accumulation of acetylcholine at nerve synapses and causing overstimulation of nerve cells, paralysis, and ultimately death in the targeted pests. Due to its effectiveness against a wide range of pests, profenofos has been extensively used in agricultural pest management practices. However, like other organophosphate pesticides, profenofos poses risks to human health and the environment.

CHAPTER 2

2. LITERATURE SURVEY

2.1 NEGATIVE IMPACT OF PESTICIDES

2.1.1 Human health impacts

Numerous studies have documented the adverse effects of pesticides on human health. Exposure to pesticides has been linked to various health problems, including cancer, reproductive disorders, neurodevelopmental disorders, and respiratory issues (Alavanja *et al.*, 2004; Mostafalou and Abdollahi, 2017). Children and pregnant women are particularly vulnerable due to their developing bodies and increased susceptibility to toxic chemicals (Landrigan *et al.*, 2017). Furthermore, pesticide residues in food and water sources can pose significant risks to human health, even at low levels of exposure (Jurewicz and Hanke, 2018).

2.1.2 Environmental impacts

Pesticides can also have detrimental effects on wildlife and ecosystems. Non-target organisms, such as bees, birds, fish, and amphibians, are often unintentionally exposed to pesticides, leading to population declines and biodiversity loss (Goulson, 2013; Gibbons *et al.*, 2015). Additionally, pesticides can contaminate soil, water bodies, and air, causing long-term damage to terrestrial and aquatic ecosystems (Lopes *et al.*, 2012; Kattwinkel *et al.*, 2011). Runoff from agricultural fields and improper disposal of pesticide containers contribute to pollution and ecological degradation (Aktar *et al.*, 2009).

2.1.3 Regulatory and policy considerations

Despite growing concerns about the negative impacts of pesticides, regulatory frameworks vary widely across countries, and enforcement of pesticide regulations can be inconsistent (Pimentel *et al.*, 2007). Efforts to mitigate pesticide risks include the development of integrated pest

management strategies, promotion of organic farming practices, and implementation of stricter

regulations on pesticide use and registration (Pretty, 2018; Sánchez-Bayo and Wyckhuys, 2019).

2.2 ALTERNATIVE APPROACHES FOR PESTICIDES

A range of alternative approaches to pesticide use have been proposed. Azzouz (2021) suggests the use of low-cost oxidative processes for treating pesticide-containing effluents, while Singh (2020) advocates for the eco-friendly and cost-effective bioremediation using microorganisms. Omar (2008) emphasizes the importance of effective pesticide application with minimal environmental impact, including the use of controlled-release formulations and precision equipment. Postel (1988) underscores the need for strategies that reduce pesticide use in agriculture and minimize waste generation in industry, such as integrated pest management and industrial waste reduction.

Debnath (2019) and Reddy (2015) both highlight the potential of functional materials and photochemical methods, respectively, for the treatment of pesticides in water. Keshavakannan (2020) emphasizes the need for alternative pest control strategies in agriculture, such as host plant resistance and bio-control, to mitigate the negative effects of pesticides on human health and the environment. Cara (2015) discusses the use of low-cost adsorbents for pesticide removal, underscoring the importance of field-scale testing and the modification of biosorbents for improved efficacy. Fraser (2012) explains the currently used treatment for pesticide containing wastewater such as incineration, hydrolysis and composting.

Nurika (2022) suggests training farmers in proper pesticide use, monitoring environmental quality, and using electrolyzed water treatment, sonolytic ozonation, and ozonated water to reduce pesticide residues. Ebrahimi (2018) reviews the use of new technologies such as machine vision, ultrasonic sensors, and spectrometry for variable rate application in sprayers, as well as pest identification and classification. Rajan

(2019) proposes the use of controlled release pesticides to reduce overuse and improve efficiency. These studies collectively underscore the potential of various alternative approaches for pesticide use, from treatment methods to pest control strategies.

2.3 PROFENOFOS PESTICIDE

Profenofos is an organophosphate pesticide widely used in agriculture, with potential toxic effects on both the environment and human health (Silva, 2013; Kushwaha, 2016). It has been found to have a synergistic effect when combined with chlorantraniliprole, another pesticide (Mishra, 2015). The pesticide has been shown to downregulate male-specific cytochrome P450 enzymes, potentially disrupting endocrine function (Moustafa, 2008). Chemically, it belongs to the group of organophosphorus compounds, characterized by their mode of action targeting the nervous system of pests. Sabat (2014) demonstrated that increasing concentrations of profenofos led to a reduction in the morphological traits and pigments of *Vigna radiata* L. seedlings. This was further supported by Mishra (2015), who found that the biochemical parameters of the seedlings were also significantly affected.

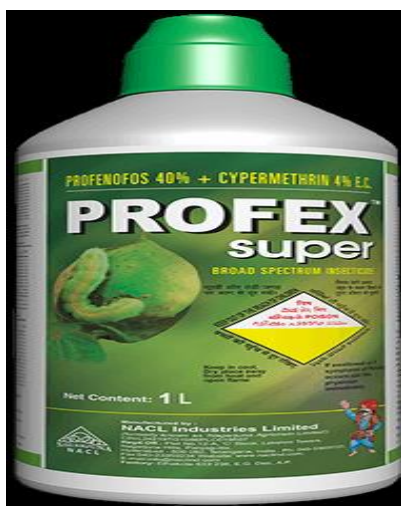


Fig 2.1: Profex pesticide

Chawla (2019) highlighted the negative effects of profenofos on soil quality indicators, such as microbial populations and enzymatic activities, with higher concentrations leading to a reduction in these indicators.

However, Jiachun (2011) proposed a compound pesticide containing profenofos, which showed a synergistic effect and good control of certain pests. However, like other organophosphate pesticides, profenofos poses risks to human health and

the environment. Exposure to profenofos can occur through inhalation, ingestion, or dermal contact during its handling, application, or consumption of contaminated food and water (Silva, 2013). Chronic exposure to profenofos has been associated with various health effects in humans, including neurotoxicity, respiratory problems, reproductive disorders, and carcinogenic potential (Moustafa, 2008).

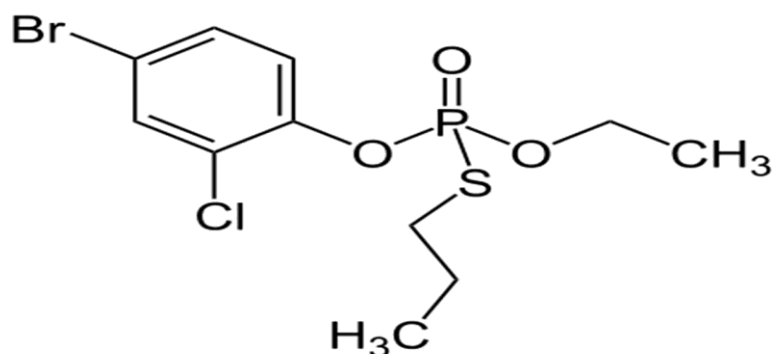


Fig 2.2: Chemical structure (2-D) of profenofos (Barun and David, 1995)

In response to growing concerns about the adverse effects of profenofos on human health and the environment, regulatory authorities in many countries have imposed restrictions on its use or banned it altogether. Alternatives to profenofos, such as integrated pest management (IPM) practices, biopesticides, and other reduced-risk pesticides, are being promoted to minimize reliance on hazardous pesticides and promote sustainable agricultural practices. Additionally, research efforts continue to focus on developing safer and more environmentally friendly alternatives to profenofos for effective pest management while minimizing associated risks (Barun and David, 1995).

TABLE 2.1: Chemical and physical properties of profenofos

(Peter et al., 2015)	
Chemical formula	C ₁₁ H ₁₅ BrClO ₃ PS
IUPAC Name	4-bromo-2-chloro-1-[ethoxy(propylsulfanyl)phosphoryl]oxybenzene
Molar mass	373.63 g/mol
Appearance	Pale yellow to amber liquid

2.4 DEGRADATION OF PROFENOFOS PESTICIDE

The degradation of profenofos, an organophosphate pesticide, has been studied through various mechanisms. Malghani (2009) identified a *Pseudomonas aeruginosa* strain that degrades profenofos through a hydrolysis mechanism, with 4-bromo-2-chlorophenol as a key metabolite. Chen (2015) found that UV/ferrate(VI) oxidation of profenofos is pH-dependent and can be enhanced by UV254, leading to the formation of O-4-bromo-2-chlorophenyl O,S- dihydrogen phosphorothioate. Mansourieh (2015) optimized the degradation of profenofos using bimetallic Fe/Ni nanoparticles, achieving a removal rate of 94.51%. Angthararuk (2017) identified degradation products of profenofos in water, including those resulting from the replacement of bromine and hydrogen by hydroxyl groups and the rupture of various chemical bonds. These studies collectively provide insights into the diverse degradation mechanisms of profenofos.

Profenofos, an insecticide, can be degraded through both biotic and abiotic pathways. Verma (2021) and Soares (2021) both identified the biodegradation of profenofos by microbial strains, with Verma proposing a degradation pathway involving an organophosphorus hydrolase enzyme. Angthararuk (2017) further explored the degradation products of profenofos, identifying two main degradation pathways involving the replacement of bromine and hydrogen by hydroxyl functional groups, and the

rupture of specific chemical bonds. Subsanguan (2019) investigated the aerobic and anoxic degradation of profenofos by *Pseudomonas plecoglossicida* strain PF1, demonstrating its

potential for bioremediation in contaminated environments. These studies collectively highlight the potential for both biotic and abiotic pathways in the degradation of profenofos.

Burkhard (1979) found that it is readily degraded under artificial sunlight conditions, with the rate of degradation being greater in moist soil. Tariq (2018) and Samara (2019) both explored the use of different methods to enhance its degradation, with Tariq focusing on reductive transformation using Fe/Ni bimetallic nanoparticles and Samara on photocatalytic degradation using silver- platinum-doped zeolite. Both studies reported significant increases in degradation efficiency. Mansouriieh (2015) further optimized the degradation of profenofos using bimetallic Fe/Ni nanoparticles, achieving a removal rate of

94.51%. The degradation products of profenofos have been identified, with the main pathways involving the replacement of bromine and hydrogen by hydroxyl functional groups, and the rupture of certain chemical bonds (Angthararuk 2017). Lastly, the sorptive and degradative assessments of the fungicide Fuberidazole in soil have been studied, with the results indicating a medium sorptive capacity and a lower risk of groundwater contamination through leaching (Aftab 2021). Profenofos undergo aerobic and anoxic degradation by *P. plecoglossicida* strain PF1, with the former being more effective (Subsanguan 2019). The bacteria *Bacillus cereus* and *Aneurinibacillus migulanus* have also been identified as effective in degrading profenofos in soil (Palanimanickam 2018).

2.5 BACTERIA AS BIOCATALYST IN BIOREMEDIATION

Bacteria were used as biosorbents because of their small size, their ubiquity, their ability to grow under controlled conditions and their resilience to a wide range of environmental situations. Some bacteria which may be used for bioremediation in heavy metals contaminated environment. They can remove heavy metals from contaminated sites either by bioaccumulation, precipitation or biosorption (Urrutia, 1997). Bacteria play a crucial role in the

bioremediation of pesticide-contaminated environments, offering a cost-effective and environmentally sound solution (Umadevi, 2017). Ming (2003) and Hassen (2020) both highlight the effectiveness of biodegradation bacteria in pesticide-

polluted soil, with the latter emphasizing the potential of rhizobacteria and their metabolites in agricultural soils. Sarker (2021) further underscores the importance of microorganisms and their enzymes in the sustainable remediation of pesticide-contaminated sites, despite the challenges of inconsistent performance and adaptation under varying conditions. Giri *et al.*, (2020) identified that *Bacillus sp.* and *Pseudomonas sp.* are used for bioremediation of pesticides from soil/water matrixes. As suggested by Fomina and Gadd (2014), the uptake of heavy metals by microbial cells through biosorption mechanisms can be classified into metabolism-independent biosorption, which mostly occurs on the cells exterior and metabolism-dependent bioaccumulation, which comprises sequestration, redox reaction, and species-transformation methods. Bioaccumulation depends on a variety of chemical, physical, and biological mechanisms and these factors are intracellular and extracellular processes, where biosorption plays a limited and ill-defined role. Various microorganisms such as bacteria, yeast, fungi, algae, and plants have been reported to tolerate and remove harmful pesticides from the environment. The advantages of using microbes for bio-remediation include natural occurrence, cheap production, easy availability to treat large volumes of wastewater due to rapid kinetics and high selectivity in terms of removal and recovery of specific pesticides.

TABLE 2.2: Different soil bacteria species capable of degrading various

pesticides (Jayaweera et al., 2022).

BACTERIAL SPECIES	TYPE OF PESTICIDE	REFERENCES
	DEGRADED	
<i>Flavobacterium spp</i>	Diazinon, Parathion	Sethunathan and Yoshida,1973
	Diazinon	Yasouri,2006
	Pentachlorophenol	Saber and Crawford,1985
	Pentachlorophenol	Briglia et al.,1990
<i>Sphingobium spp</i>	Fenobucarb, Carbaryl	Kim et al.,2014
<i>Sphingobium fuliginis</i>	Diazinon, Parathion	Kawahara et al.,2010
<i>Sphingomonas spp</i>	Buprofezin	Liu et al.,2015
<i>Agrobacterium spp</i>	Fenvalerate	Yu et al.,2013
	Diazinon	Yasouri,2006
	Methylparathion, Phoxim, Methamidophos,	Wang et al.,2012
	Chlorpyrifos, Carbofuran, Delta methrin	
	Atrazine	Struthers et al.,1998
<i>Pseudomonas spp</i>	Diazinon	Yasouri,2006 ;
	Profenofos	Cyco et al., 2009 Malghani et al.,2009
<i>Pseudomonas aeruginosa</i>	Methyl Parathion	Chaudhry et al.,1988
<i>Pseudomonas alcaligenes</i>	<i>Pseudomonas frederiksbergensis</i>	Chlorpropham, Chlorobutylamine, Isopropyl N-phenylcarbamate, Methyl N-(3,4-dichlorophenyl) carbamate, propanil
<i>Pseudomonas psychrophila</i>		Chlorpyrifos, Cypermethrin, Endosulfan
<i>Pseudomonas</i>		

Endosulfan, Chlorpyrifos, Malathion	Marty and Vouges, 1987	
	Naphade <i>et al.</i> , 2012	
	Iqbal and Bartakke, 2014	
<i>Serratia spp</i>	Diazinon	Cycoř <i>et al.</i> , 2009

<i>Serratia liquefaciens</i> and <i>Serratia marcescens</i>	Diazinon, Malathion, Chlorpyrifos	Iqbal and Bartakke, 2014
<i>Enterobacter spp</i> (Strain B14)	Chlorpyrifos, Parathion, Diazinon, Coumaphos, Isazofos	Singh et al., 2004
<i>Burkholderia gladioli</i>	Profenofos Dimetoate	Malghani et al., 2009 Iqbal and Bartakke, 2014
<i>Rhodococcus chlorophenolicus</i>	Endosulfan	Kumare et al., 2007

Bacillus spp

Mesotrione

Batisson et al., 2009

CHAPTER 3

3. AIM AND OBJECTIVES

3.1 AIM

To isolate, identify and characterize the pesticide degrading bacteria for bioremediation.

3.2 OBJECTIVES

To isolate cultivable microbes from soil collected from “Sethuammal Farms’ Thirunelveli.

To identify the most potent pesticide degrading bacteria that can grow in the presence of pesticides.

To evaluate the efficacy of isolated bacteria in degrading pesticides on liquid medium.

To investigate the beneficial aspects of isolated bacteria for bioremediation.

To develop a method that evaluates the degradation of pesticides in intact columns of core soil.

CHAPTER 4

4. MATERIALS AND METHODS

4.1 COLLECTION OF SOIL SAMPLES

The soil samples used were collected from Sethuammal farm, located at Thirunelveli district of Tamil Nadu. Aubergine (*Solanum melongena*) and Okra (*Abelmoschus esculentus*) are the crops grown in the farm. These crops have been sprayed with profenofos organophosphate insecticide for the last 6-7 months. Pesticide contaminated soil samples were collected at three different points in the sampling location, 100 metres apart from each point following sampling procedure.

4.2 MEDIA PREPARATION

Fine powdered 1g of soil samples was weighed and mixed with 99 ml of sterile distilled water. Then the mixture kept at 150 rpm shaking for one hour in order to facilitate the complete mixing. Nutrient agar was taken as a medium for culturing the bacterial species present in the soil sample. Nutrient medium composed of 0.5 % peptone (organic nitrogen source), 0.3% beef extract/yeast extract (provides vitamins, carbohydrates, nitrogen and salts), 0.5% sodium chloride (osmotic regulation) and 1.5 % agar (solidifying agent). After added all the essential nutrients, the pH of the medium was adjusted to neutral level (7-7.4) at 25° C. Finally, one ml of profenofos 40% (an organophosphate and a member of monochlorobenzenes) pesticide at 1.5 ul concentration was added to the medium and mixed thoroughly. The medium dispensed into flasks and autoclaved at 121°C for 15 minutes. After autoclaved, the medium allowed to get cool for 55°C and poured into petriplates.

4.3 ISOLATION OF ORGANOPHOSPHATE DEGRADING BACTERIA

The specific amount of soil sample was weighed (0.25g) from each four soil samples and the total 1g of soil was dissolved in 90ml of distilled water.

Then the sample was kept in orbital shaker at 150 rpm for 2 hours for complete mixing. After proper dissolving the sample was serially diluted upto 10^{-9} and 0.1 ml of the sample from all the dilutions were spreaded on solidified nutrient agar plates. After incubation, plates were observed for different isolates based on morphological traits.

4.4 GROWTH KINETICS IN LIQUID MEDIA

The isolated colonies were checked for their growth in LB (Luria Bertani) broth composed of 0.5 % tryptone (organic nitrogen source), 0.3% beef extract/yeast extract (provides vitamins, carbohydrates, nitrogen and salts), 0.5% sodium chloride (osmotic regulation) containing 2.5 μ l concentration of profenofos pesticide.

4.5 IDENTIFICATION OF PESTICIDE DEGRADING BACTERIA

The most efficient bacterial species which showed better growth on liquid media and on increasing the concentration of pesticide was isolated and identified by various tests such as gram staining, motility test and biochemical test etc. Gram staining was done by following the standard method. Motility was performed by following hanging drop method. Certain biochemical studies such as Indole, methyl red, Voges Proskaur, citrate utilization, catalase, sugar utilization tests and phosphate solubilisation test also performed by using standard methods.

4.6 SCREENING OF BACTERIAL PESTICIDE UTILIZER

Screenings of isolates having degradation ability were screened on the basis of degradation of profenofos. Bacteria with greater degrading activity were chosen, and these were used in subsequent analyses. Zone of clearance was used to measure the isolates' ability to degrade profenofos.

4.7 QUANTIFICATION OF PESTICIDE RESIDUE

This was carried out using high proficiency liquid chromatography on the biodegraded sample. The samples were analysed by directly derivatizing an

aliquot and the derivatizing reagent mixture was prepared fresh by mixing one volume of acetonitrile with two volumes of monobromobimane.

4.8 AQUEOUS EXTRACT OF THE ISOLATED SAMPLE

Aliquots 1.6 ml of the derivatization reagent mixture were added to 2 ml HPLC vials and were sealed using screw caps. The vials were then cooled to about -4°C before proceeding. An eppendorf tube was used to add a aliquot of sample extract. Analyte derivatization was then performed by heating the reaction vial to 95°C for one hour, then cooled to room temperature. The excess derivatization reagents were evaporated. The residue was dissolved in 200 μl of ethyl acetate, capped and saved for HPLC analysis.



Fig 4.1: Aqueous extracts of the sample

4.9 HIGH PROFICIENCY LIQUID CHROMATOGRAPHIC ANALYSIS OF THE SAMPLE EXTRACT

Chromatographic analysis was performed using a high proficiency liquid chromatography, equipped with a split injector and an auto sampler. The chromatographic conditions were as follows: HP-LC (95 CAN 5 H₂O) column [50-

150 mm in length ; 4.6 mm in diameter(inner)], water mixed with an organic modifier acetonitrile 20% [polar] was used for mobile phase, with a constant flow (1.2 ml/min) and silica based material was used for stationary phase, injection

volume (20 ul), injector temperature was 40°C, HPLC temperature run: 50°C - 70°C for 30 mins. The analytes were quantified using the external standard method with calculations based on peak height. Analyte quantification was carried out with HPLC-UV software.

4.10 MOLECULAR LEVEL IDENTIFICATION OF BACTERIAL ISOLATE

4.10.1 Bacterial DNA extraction

50-100 mg bacterial cells that have been resuspended in 200 ul of water or isotonic buffer (e.g.PBS) or up to 200 mg of tissue were added to a ZR bashingbead lysis tube containing 750 ul Lysis Solution. It was secured in a bead fitted with 2ml tube holder assembly and processed at maximum speed for

> 5 minutes. The ZR bashingbead lysis tube was centrifuged in a microcentrifuge at > 10,000 x g for 1 minute. 400 ul supernatant was transferred to a centrifuge tube and centrifuged at 7,000 x g for 1 minute. 1,200 ul of Bacterial DNA Binding Buffer was added to the filtrate in the centrifuge tube. 800 ul of the mixture was transferred to a Zymo-Spin TM IIC column in a collection tube and centrifuged at 10,000 x g for 1 minute. The Zymo-Spin TM IIC column has a maximum capacity of 800 ul. The flow was discarded through the collection tube. 200 ul of DNA pre-wash buffer was added to the Zymo-Spin TM IIC column in new collection tube and centrifuged at 10,000 x g for 1 minute. 500 ul bacterial DNA wash buffer was added to the Zymo-Spin TM IIC column and centrifuged at 10,000 x g for 1 minute. The Zymo-Spin TM IIC column was transferred to a clean 1.5 ml microcentrifuge tube and 100 ul (35ul) DNA elution buffer added directly to the column matrix. It was then centrifuged at 10,000 x g for 30 seconds to elute the DNA.

4.10.2 Quantity and quality check

The product was checked on a nanodrop of model 2000 from ThermoScientific to quantify the concentration of the extracted DNA and also determine purity by measuring at 260/280 nm amount of proteins left in the DNA extract.

4.10.3 Polymerase Chain Reaction (PCR) analysis

The DNA was subjected to a cocktail mix and condition for the PCR. PCR reaction cocktail consisted of 10 ul of 5 x Go Taq colourless reactions, 3 ul of MgCl₂, 1 μ l of 10 mM of dNTPs mix, 1 ul of 10 pmol each. The Internal Transcribed Spacer (ITS) gene for characterization of bacteria, ITS universal primer set which flank the 16S was used.

- 16SF: GTGCCAGCAGCCGCGCTAA

- 16SR: AGACCCGGGAACGTATTAC

Polymerase Chain Reaction (PCR) conditions include a cycle of initial denaturation at 94 C for 5 minutes, followed by 35 cycles of each cycle comprised of 30 seconds denaturation at 94 C, 30 seconds annealing of primer at 55 C, 1.5 minutes extension at 72 C and a final extension for 7 minutes at 72 C.

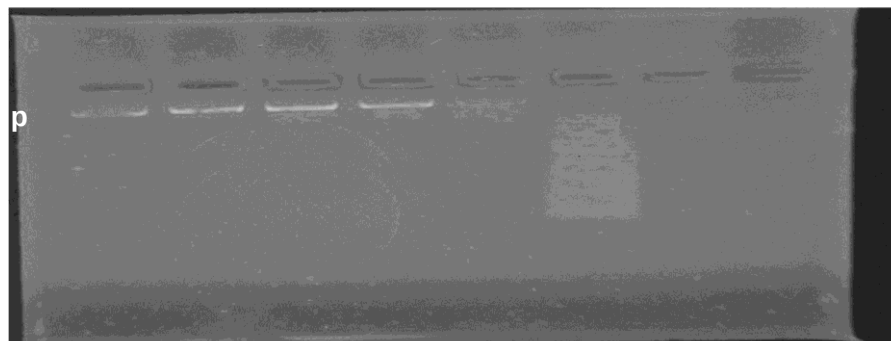


Fig 4.2: Agarose gel electrophoresis of PCR product

4.11 PURIFICATION OF AMPLIFIED PCR PRODUCT

The amplified fragments were purified with ethanol in order to remove the PCR reagents. Briefly, 7.6 ul of sodium acetate 3M and 240 ul of 95% ethanol were added to each about 40 ul PCR amplified product in a new sterile

1.5 ul eppendorf tube, mixed thoroughly by vortexing and kept at -20°C for at least 30

minutes. Centrifugation was done for 10 minutes at 13000 Revolution per minute and 4°C followed by removal of supernatant (tube was inverted on tissue once) after which the pellet were washed by adding 150 ul of 70 % ethanol and mixed, then centrifuged for 15 minutes at 7500g and 4°C. Again all supernatant was removed and tube inverted on paper tissue and allowed to dry in the fume hood at room temperature for 10-15 minutes. It was re-suspended with 20 ul of sterile distilled water and kept in -20 C prior to sequencing. The purified fragment was checked on a 2% Agarose gel ran on a voltage of 120 V for about 1 hour, to confirm the presence of the purified PCR product. The ladder used for bacteria is hyper ladder 1 from BioLone while 1 kb plus ladder from Invitrogen was used for bacteria. The expected base pair of the amplicon is around 850 bp. The amplified samples were then sent for sequencing.

4.12 GENOME SEQUENCING AND ANNOTATION

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from applied biosystems using manufacturer' manual while the sequencing kit used was that of big dye terminator v3.1 cycle sequencing kit. Bio-edit software and MEGA 6 were used for all genetic analysis. The classification/identification of organisms was performed by a local nucleotide BLAST search against the non-redundant version of the NCBI ref database.

CHAPTER 5

5. RESULTS AND DISCUSSION

Harmful pesticides are present in water, agricultural soil, sediments, etc and they have both natural and anthropogenic origin. Anthropogenic sources generate a constant and permanent increasing pollution, while the natural source is usually a seasonal phenomenon, influenced by weather, and generally does not generate pollution. Hence, it is indispensable to eradicate the pesticide pollution for the safety of living things. In view of this, the present study is designed to isolate microbes (bacteria) from the pesticide contaminated soil and its pesticide degrading capacity was analysed.

5.1 SAMPLE COLLECTION

The sample collection area, Sethuammal farm is one of the largest farm of Thirunelveli which involved in the utilization of various harmful pesticides. Hence, the area was chosen for the present study. Soil samples collected from three different points of the study area were mixed completely to retrieve all the bacteria present in the sample. In preliminary studies, even in higher dilution, numerous colonies were obtained. Hence to minimize the number of colonies, diluted soil samples were directly inoculated on pesticide supplemented medium.

5.2 ISOLATION OF PESTICIDE DEGRADING / TOLERANT BACTERIA

The sample was diluted upto 10^{-9} and the higher dilutions were used for bacterial isolation. Among three dilutions, highest dilutions showed lesser colonies which are easy to isolate. Lower dilutions produced more number of colonies with different morphological appearance. Some colonies were creamy in nature, some of them had glassy appearance. Yet, colonies which appeared in highest concentration of pesticides were considered for further studies.

In case of cypermethrin, 7, 6, 4 and 3 colonies were observed in 0.5, 1, 2 and 2.5 ul concentrations. The highest concentration (3 ul) didn't produce any

colonies. The higher concentration is more toxic would be the possible explanation. About 3 colonies obtained in diazinon supplemented medium where as only one colony was obtained in profenofos supplemented medium. Totally

20, 18 and 12 colonies were obtained in cypermethrin, diazinon and profenofos supplemented medium which suggested that profenofos is more toxic than other two pesticides. The obtained 12 colonies from profenofos supplemented media were streaked individually on nutrient agar plates and used for remaining studies.

TABLE 5.1: Physical and biochemical characteristics of the subcultured colonies

Bacterial isolates	Colour	Gram reaction	Cellular morphology	Catalase test	MR-VP	Indole	Phosphate solubilisation test	Citrate utilisation test
PS1	Cream	+	Rod	+	-	-	+	-
PS2	Cream	-	Cocci	-	-	-	+	-
PS3	Yellow	+	Cocci	-	-	-	-	-
PS4	White	+	Rod	-	-	-	-	+
PS5	White	+	Rod	+	-	-	-	-
PS6	Cream	+	Cocci	+	+	-	+	-
PS7	Yellow	-	Cocci	+	+	-	-	+
PS8	Cream	-	Rod	+	+	-	-	+
PS9	Cream	+	Rod	-	-	-	-	+
PS10	Cream	+	Cocci	+	-	-	+	-
PS11	White	+	Cocci	+	-	+	-	-
PS12	Cream	+	Rod	+	-	-	+	+

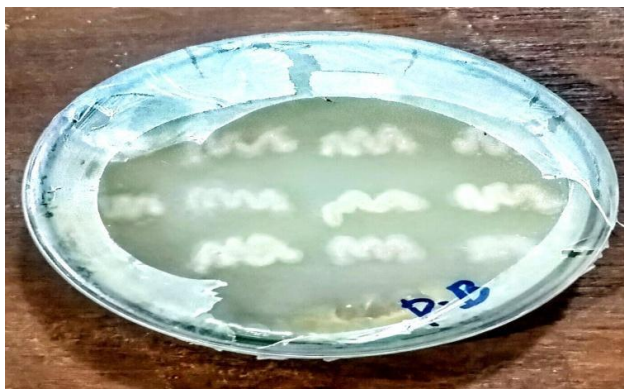


Fig 5.1: Subcultured bacterial isolates obtained from pesticide supplemented media (12 colonies)

5.3 GROWTH KINETICS OF THE ISOLATED BACTERIAL COLONIES

Totally 12 colonies were isolated from the soil sample. They were further analysed for their growth kinetics when they were challenged with pesticides. During initial time incubation, the growth parameters were not much differed among all organisms. When bacterial cells entered into log phase a drop in growth was observed in pesticide supplemented medium. Bacterial cells which inoculated in profenofos medium were affected much when compared to other pesticides. Yet the growth reduction was retrieved within next 4 hrs by the isolated bacterial colonies which confirmed that they were tolerant to pesticides used in the present study. In the experiment conducted for studying the pesticide degrading potential of the isolated bacterial colonies in pesticide amended broth medium, it was observed that PS1 isolate has efficacy in tolerating with varied potential. PS1 showed highest scavenging efficacy which tolerated and maintained the steady growth upto 24 hrs while other colonies tolerated upto 12 hrs only.

5.4 PRELIMINARY IDENTIFICATION OF THE BACTERIAL ISOLATE

Since the isolated bacterial species holds much promise in the field of bioremediation, efforts had been made to identify the organism at basic and molecular level. In initial screening, the bacteria was determined to be a

gram positive, motile, facultatively anaerobic rod shaped bacteria which generated a

mucoïd colony on nutrient agar. In addition, it was positive for catalase and phosphate solubilisation test negative for indole production, MR-VP, citrate test and sugar utilisation test. Based on these characteristics, the isolate was identified as *Fredinandcohnia salidurans*. The genus *Fredinandcohnia* is a group of gram-positive motile rods known for their plant growth promoting activities.

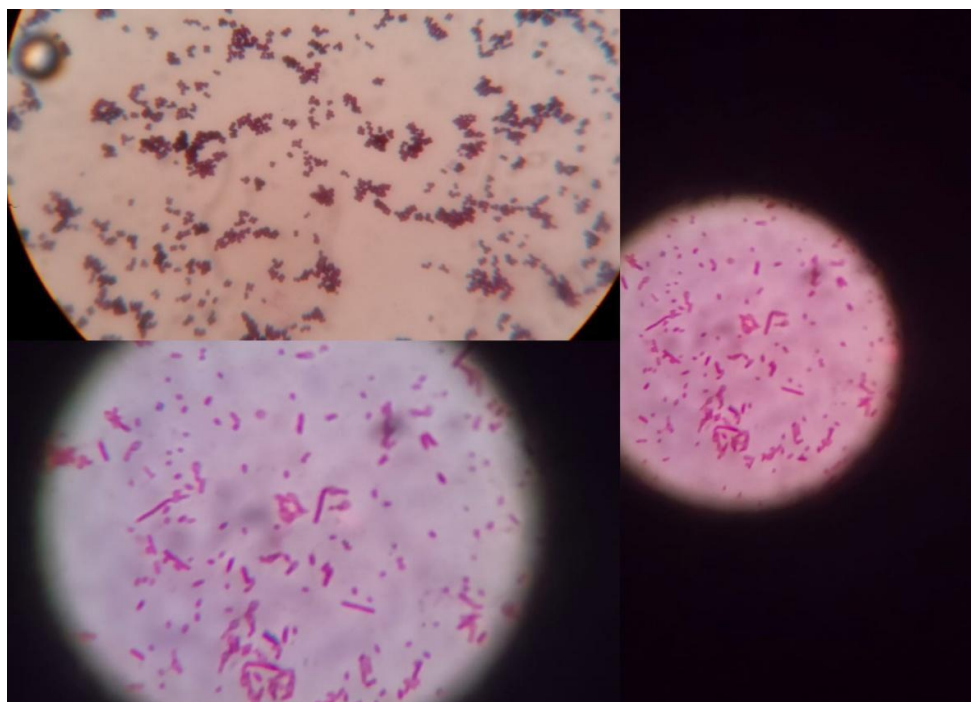
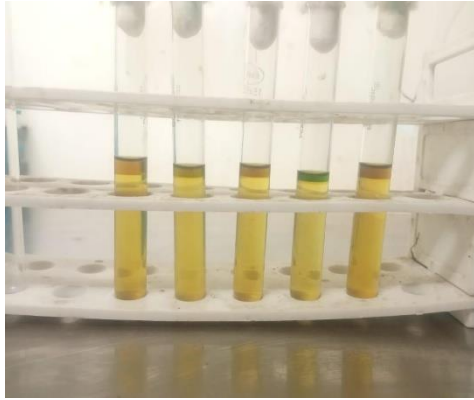


Fig 5.2: Gram staining showed Positive bacilli shaped bacteria

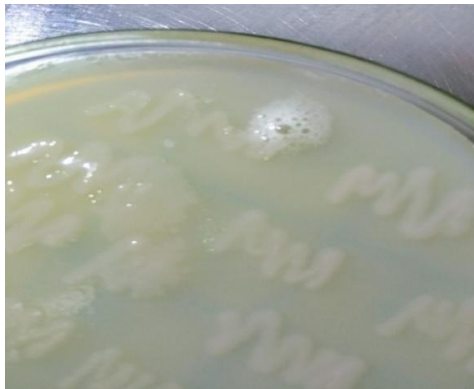


Indole negative

positive

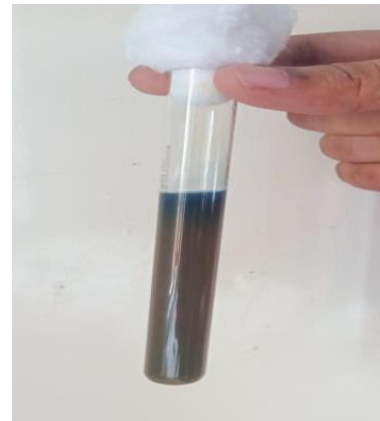


MR-VP



Catalase positive

negative



Citrate

Fig 5.3: Biochemical reactions (IMViC)



Fig 5.4: Phosphate solubilization test (Positive)

5.5 HIGH PROFICIENCY LIQUID CHROMATOGRAPHIC ANALYSIS OF THE ISOLATE

In this study; gram positive, rod shaped *Fredinandcohnia salidurans* bacteria had degraded the pesticide profenofos by 70% at 2.5 ul concentration. Several bacteria such as *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Serratia marcescens*, *Enterobacter aerogenes*, *Acinetobacter calcoaceticus*, *Alcaligenes faecalis* have been reported to degrade profenofos with an average degradation of 60-80%. Additionally, the effectiveness of degradation can depend on factors such as the concentration of profenofos, temperature, pH, and the presence of other pollutants or organic matter in the environment.

SAMPLE INFORMATION			
Sample Name:	profenofos sample	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	
Vial:	1:a,2	Acq. Method Set:	Profenofos 95 ACN 5 H
Injection #:	2	Processing Method:	profenofos sample
Injection Volume:	20.00 ul	Channel Name:	270.0nm
Run Time:	30.0 Minutes	Proc. Chnl. Descr.:	PDA 270.0 nm
Date Acquired:	02-Apr-24 2:23:21 PMIST		
Date Processed:	02-Apr-24 3:44:39 PMIST		

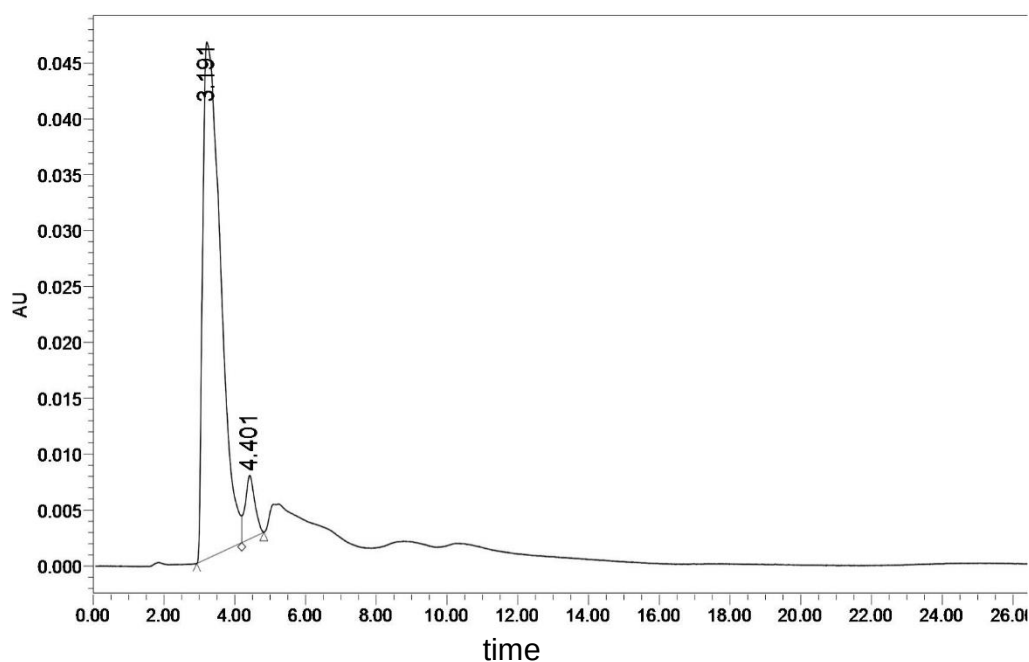


Fig 5.5: Degradation graph of profenofos sample in the presence of isolated bacteria

SAMPLE INFORMATION

Sample Name:	profenfos standard re	Acquired By:	S y s t e m
Sample Type:	Unknown	Sample Set Name:	
Vial:	1:a,2	Acq. Method Set:	Profenofos 95 ACN 5 H
Injection #:	3	Processing Method:	profenfos
Injection Volume:	20.00 ul	Channel Name:	270.0nm
Run Time:	30.0 Minutes	Proc. Chnl. Descr.:	PDA 270.0 nm
Date Acquired:	02-Apr-24 2:58:08 PM IST		
Date Processed:	02-Apr-24 3:41:05 PM IST		

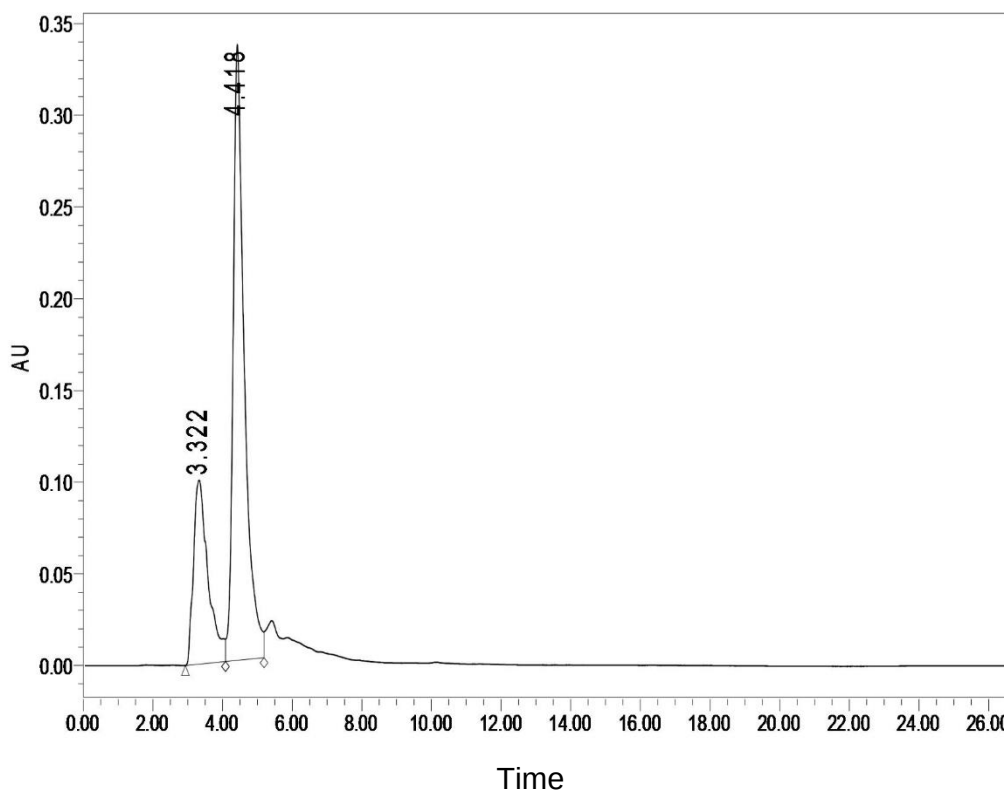


Fig 5.6: Degradation graph of profenofos sample in the absence of isolated bacteria

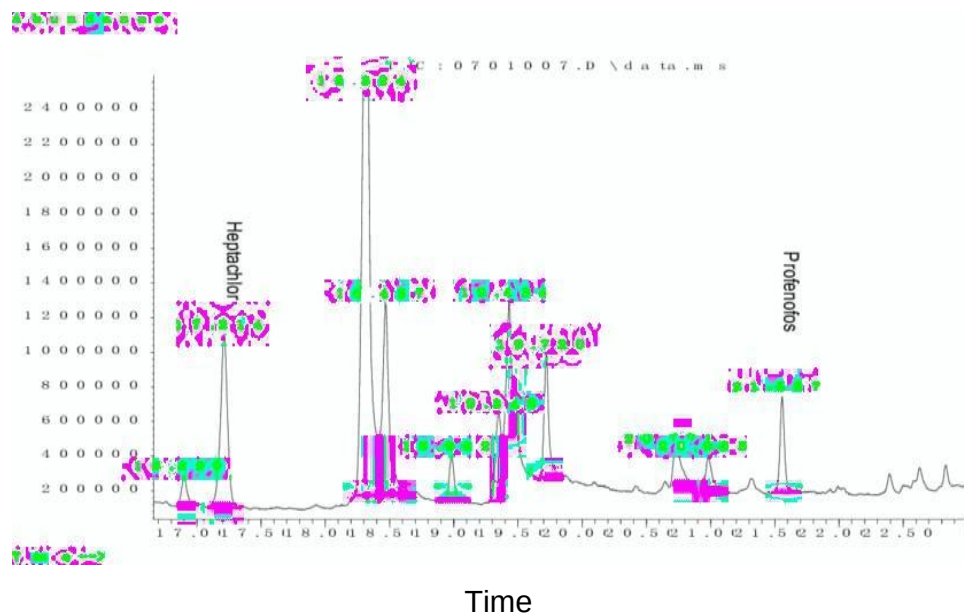


Fig 5.7: Standard cocentration of profenofos pesticide

The pesticide contaminated soil sample has been inoculated with the isolated bacteria for a month in a test tube (TS1) and tested for its biodegradation capacity. In order to compare the degradation capacity, another test tube was taken, containing pesticide contaminated soil without culture (TS2). Both were sent for HPLC analysis, as a result TS1 showed maximum degradation by 70% whereas, TS2 showed only 10% of degradation.

5.6 MOLECULAR LEVEL IDENTIFICATION OF THE BACTERIAL ISOLASTE

Further the species was characterized using 16SrRNA sequencing. The results indicated that the bacterium belongs to the category of *Bacillus spp.* The BLAST analysis of sequences showed 99 to 96.6 % of similarity with *Fredinandcohnia salidurans*. The identified bacteria had pesticide degrading property as indicated in HPLC study. *F. salidurans* bacteria, though not as extensively studied as some other microbial species, offer several potential applications and uses in various fields, particularly in agriculture and environmental remediation. *F. salidurans* has demonstrated a

remarkable ability to degrade a wide range of organic pollutants and contaminants present in soil and water environments. These bacteria can metabolize aromatic compounds, hydrocarbons, phenols, and other organic pollutants through enzymatic

degradation pathways. Their application in bioremediation processes can help to detoxify contaminated soils and water bodies, contributing to environmental cleanup efforts. In agricultural settings, *F. salidurans* can play a crucial role in soil restoration by breaking down pollutants and improving soil quality. They possess mechanisms for metal tolerance and can aid in the remediation of soils contaminated with heavy metals, such as cadmium, zinc, and lead. By facilitating the breakdown of organic pollutants and metals, these bacteria contribute to restoring soil fertility and promoting sustainable agriculture practices (Gupta *et al.*, 2020). *F. salidurans* bacteria can also enhance soil fertility by decomposing organic matter and releasing nutrients such as nitrogen, phosphorus, and sulfur into the soil. Their metabolic activities contribute to nutrient cycling processes, making essential nutrients more readily available to plants and promoting healthy soil ecosystems. Application of these bacteria in agricultural soils can reduce the reliance on chemical fertilizers, leading to more sustainable and environmentally friendly farming practices (Gupta *et al.*, 2020). Research suggests that *F. salidurans* may have potential as biofertilizers due to their ability to solubilize phosphorus and fix atmospheric nitrogen. As biofertilizers, these bacteria could enhance plant nutrient uptake, improve soil structure, and promote crop growth while reducing the need for synthetic fertilizers.

F. salidurans bacteria can indirectly promote plant growth by improving soil health and nutrient availability. Their presence in the rhizosphere can stimulate plant growth through interactions such as enhanced nutrient uptake, production of growth-promoting compounds, and protection against soil-borne pathogens. Beyond agriculture, *F. salidurans* has applications in environmental biotechnology, including the treatment of industrial wastewater and polluted water bodies. Their ability to degrade diverse pollutants makes them valuable in the bioremediation of contaminated environments, contributing to ecosystem restoration and protection (Gupta *et al.*, 2020). Further research and development efforts are needed to optimize their use and fully harness their capabilities for sustainable resource management and environmental protection.

Descriptions	Graphic Summary	Alignments	Taxonomy					
Sequences producing significant alignments								
Download Select columns Show 100								
select all 100 sequences selected								
GenBank Graphics Distance tree of results MSA Viewer								
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☒ Fredinandcoohnia salidurans strain KNUC7312 16S ribosomal RNA gene, partial sequence	Fredinandcoohnia salidurans	1803	1803	100%	0.0	97.37%	1481	KX904715.1
☒ Fredinandcoohnia salidurans strain KNUC7312 16S ribosomal RNA, partial sequence	Fredinandcoohnia salidurans	1803	1803	100%	0.0	97.37%	1481	NR_179035.1
☒ Bacillus sp. 5.5LF 38T partial 16S rRNA gene, strain 5.5LF 38T	Bacillus sp. 5.5LF 38T	1803	1803	100%	0.0	97.37%	1452	FM958155.1
☒ Fredinandcoohnia salidurans strain 24502 16S ribosomal RNA gene, partial sequence	Fredinandcoohnia salidurans	1797	1797	99%	0.0	97.45%	1407	OR430296.1
☒ Bacillus sp. 210_44 16S ribosomal RNA gene, partial sequence	Bacillus sp. 210_44	1797	1797	99%	0.0	97.45%	1457	GQ199746.1
☒ Fredinandcoohnia salidurans strain V5352 16S ribosomal RNA gene, partial sequence	Fredinandcoohnia salidurans	1794	1794	99%	0.0	97.45%	1421	PP256870.1
☒ Bacillus sp. (in: Bacteria) strain VITAPRRKCU-2 16S ribosomal RNA gene, partial sequence	Bacillus sp. (in: firmicutes)	1792	1792	100%	0.0	97.19%	1536	MH118520.1
☒ Bacterium strain GIm38 16S ribosomal RNA gene, partial sequence	bacterium	1792	1792	99%	0.0	97.36%	1491	MH569453.1
☒ Bacillus sp. (in: Bacteria) strain GIm38 16S ribosomal RNA gene, partial sequence	Bacillus sp. (in: firmicutes)	1792	1792	99%	0.0	97.36%	1491	MH569430.1
☒ Fredinandcoohnia onubensis strain V6387 16S ribosomal RNA gene, partial sequence	Fredinandcoohnia onubensis	1792	1792	99%	0.0	97.36%	1423	PP257308.1
☒ Fredinandcoohnia salidurans strain SSL2 16S ribosomal RNA gene, partial sequence	Fredinandcoohnia salidurans	1792	1792	100%	0.0	97.19%	1481	OR584236.1
☒ Bacillus humi strain SBB25 16S ribosomal RNA gene, partial sequence	Fredinandcoohnia humi	1792	1792	99%	0.0	97.45%	1417	KP790038.1
☒ Bacillus humi strain N12 16S ribosomal RNA gene, partial sequence	Fredinandcoohnia humi	1792	1792	100%	0.0	97.19%	1481	KP324953.1
☒ Bacillus humi partial 16S rRNA gene, isolate 0911TES10.14	Fredinandcoohnia onubensis	1792	1792	99%	0.0	97.36%	1433	LN774332.1

Fig 5.8: NCBI BLAST analysis for the selected bacterial isolate PS1

Fredinandcohnia salidurans strain KNUC7312 16S ribosomal RNA gene, partial sequence

Sequence ID: [KX904715.1](#) Length: 1481 Number of Matches: 1

Range 1: 23 to 1086 [GenBank](#) [Graphics](#)

Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
1803 bits(976)	0.0	1038/1066(97%)	12/1066(1%)	Plus/Plus
Query 1	GCCGTAATACATGCAAGTCGAGCGAACCTTTTGAGGAGCTTGCTCCCAAAAGTTAGCGGCG	60		
Sbjct 23	GCC - TAATACATGCAAGTCGAGCGAACCTTTTG - GGAGCTTGCTCCCAAAAGTTAGCGGCG	80		
Query 61	GACGGGTGAGTAACACGTGGGCAACCTGCCTATAAGACTGGGATAACTTCGGGAAACCGG	120		
Sbjct 81	GACGGGTGAGTAACACGTGGGCAACCTGCCTATAAGACTGGGATAACTTCGGGAAACCGG	140		
Query 121	AGCTAATACCGGATAACATATGAGACCACATGGTCTCATATTTAAAGATGGCTTTTAGCT	180		
Sbjct 141	AGCTAATACCGGATAACATATGAGACCACATGGTCTCATATTTAAAGATGGCTTTTAGCT	200		
Query 181	ATCACTTATAGATGGGCCCGCGGCGCATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGG	240		
Sbjct 201	ATCACTTATAGATGGGCCCGCGGCGCATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGG	260		
Query 241	CGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCA	300		
Sbjct 261	CGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCA	320		
Query 301	GACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCA	360		
Sbjct 321	GACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCA	380		
Query 361	ACGCCGCGTGAGCGAAGAAGGTCTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAG	420		
Sbjct 381	ACGCCGCGTGAGCGAAGAAGGTCTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAG	440		
Query 421	TACCGTTCAAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACG	480		
Sbjct 441	TACCGTTCAAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACG	500		

Fig 5.9: Nucleotide sequence alignment analysis for PS1 isolate

CHAPTER 6

6. SUMMARY AND CONCLUSION

6.1 SUMMARY

In this study, pesticide degrading bacteria were isolated from farm soil and screening was done to check their capacity to degrade the profenofos pesticide. Then isolated bacteria were checked against different temperature, pH, NaCl concentration for observing their effects. Also isolated bacteria were identified by their gram nature, biochemical characterization by comparing with Bergey's manual of systematic bacteriology. Twelve bacteria (PS1 – PS12) isolated by enrichment in NA medium supplemented with profenofos pesticide. On increasing the concentration only two colonies (PS1 and PS10) showed good tolerance. Whereas, only PS1 showed good growth kinetics on liquid media. Therefore, PS1 is used for further studies. Extraction of plasmid DNA and plasmid curing was done to check whether pesticide degrading bacterial gene present in chromosomal or extra-chromosomal.

In HPLC analysis, PS1 showed maximum degradation by 70%, while PS10 showed minimum degradation. According to Bergey's manual of systematic bacteriology isolated bacteria were identified as *Fredinandcohnia salidurans*. Thus, bioremediation techniques are more economical than traditional methods and pollutants can be treated on site. Other than that the risk of spread of contamination reduces making the process a safe one for dealing with very harmful xenobiotics like pesticides. Bioremediation is also very useful because the fertility of the soil that had been lost or hampered due to accumulation of pesticide for a very long period of time, thus improving the capacity of the agricultural field to meet the demands of the ever growing population efficiently. Thus, bioremediation can be used to stabilize, detoxify, or reduce the toxicity of contaminated soil.

6.2 CONCLUSION AND FUTURE PERSPECTIVES

Bioremediation of pesticides by microbes provides plenty of advantages including low input cost, long period removal process, an easy and simple requirement for equipment and space, providing economically effective environmental protection. It can play a greater role in mitigating the potential risk posed by pesticides to the natural ecosystem without losing the production and productivity in agriculture. However, the environmental pollution and health hazards caused by pesticides still remain a burning issue. This might be due to the lack of identified versatile microorganisms that can be used for detoxifying, a variety of different pesticides. Moreover, genetically modified microorganisms can offer a new hope in this regard. Furthermore, there can be a plenty of unidentified microorganisms with various pesticides detoxification capabilities. Hence, conducting further studies in order to identify new microorganisms with such capabilities in different geographical regions of the world is timely important.

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