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Isolation and Characterization of Chlorpyrifos Degrading Microorganisms from Agriculture Soil: A Review

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Abstract

Chlorpyrifos (CPF), a globally used organophosphate pesticide, has attracted the world's attention because of its persistence in soil ecosystems and its negative ecological and health effects. Microbial degradation offers a sustainable eco-friendly and cost-effective strategy to counteract CPF contamination in agricultural soils. This review offers a comprehensive overview of recent progress in the isolation and characterization of pesticide-degrading microorganisms with special emphasis on chlorpyrifos. Several bacterial genera such as Pseudomonas, Bacillus, Staphylococcus, and Alcaligenes have been found to possess outstanding CPF-degrading abilities, frequently using CPF as an exclusive carbon or phosphorus source.The review also covers field applications of CPF-degrading strains by bioaugmentation, biostimulation, and rhizoremediation, but for handling limitations in survivability of the strains and qualitative degradation. The future technologies such as whole-genome sequencing, synthetic biology, and engineering of microbial consortia are suggested to be employed for optimizing CPF bioremediation. This review highlights generally the capability of microbial technologies to facilitate sustainable pesticide management and provides a research pathway for future research in environmental bioremediation.

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Abbreviations

CPF	Chlorpyrifos
TCP	3,5,6-trichloro-2-pyridinol
KEGG	Kyoto Encyclopedia of Genes and Genomes
AI	Artificial Intelligence
CRISPR	Clustered regularly interspaced short palindromic repeats.

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Introduction

Pesticide application continues to be a central part of contemporary agriculture, with organophosphate chemicals such as chlorpyrifos (CPF) being among the most commonly applied because of their efficacy against a wide range of pests.[1] Nevertheless, extensive and unregulated use of such chemicals has resulted in tremendous ecological impacts, such as bioaccumulation, soil pollution, groundwater pollution, and toxic impacts on non-target organisms including humans.[2,3]

Chlorpyrifos is moderately persistent in the environment and can break down into toxic degradation products such as 3,5,6-trichloro-2-pyridinol (TCP), which is more water-soluble and stable than its parent.2 Microbial degradation has become a popular and low-cost method of detoxifying CPF-contaminated environments. Many bacterial strains cultured from CPF-polluted soils have been reported to possess efficient degradation potential.[4,5]

This review integrates recent studies on microbial degradation of CPF with emphasis on isolation and biochemical characterization of competent strains, mechanisms of degradation, and practical relevance to *bioremediation.*

Chlorpyrifos in Agriculture: Use and Environmental Impact

Chlorpyrifos is a general insecticide that is often utilized in the management of foliage and soil-borne insects on various crops such as cotton, maize, vegetables, and fruits.[1] It acts by inhibiting the enzyme acetylcholinesterase, an enzyme involved in nerve function of insects and vertebrates.[6]

Although its application has decreased in some nations as a result of regulatory limitations, it is still common in most developing countries. CPF's fate in the environment is multifaceted and determined by soil pH, water content, temperature, and microbial structure.[7] CPF residues have been detected in surface and groundwater, and in foodstuffs, prompting worldwide concern regarding long-term exposure.[8]

Microbial remediation becomes a primary tool not only to lower CPF environmental levels but also to recover soil quality and biodiversity negatively impacted by long-term pesticide application.[9,10]

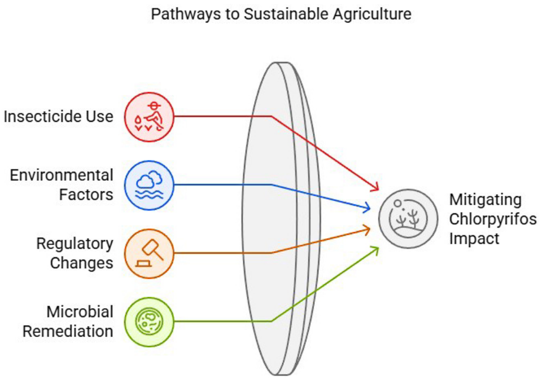

Fig. 1: Representing various pathways to sustainable agriculture.

Microbial Biodegradation of Chlorpyrifos

Biodegradation is defined as the degradation of pollutants by microbial enzymatic action, degrading toxic substances to less toxic or inert end products.[2] Soil microorganisms, especially bacteria and fungi, have the potential to utilize CPF as a carbon or phosphorus source.

Major Microbial Genera Involved Are

- *Pseudomonas*[11,12]
- *Bacillus*[13,14]
- *Staphylococcus*[4]
- *Alcaligenes*[15]
- Enterobacter and Klebsiella[16]

The efficiency of degradation usually relies on strain-specific enzymatic action, environmental conditions, and gene regulation. Hydrolases, oxidoreductases, and phosphatases are the enzymes produced by these organisms to act on CPF's chemical composition, causing detoxification.[13]

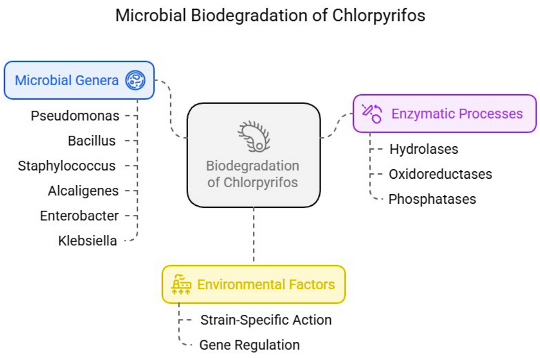

Fig. 2: Representing Microbial Biodegradation of Chlorpyrifos.

Isolation of Chlorpyrifos-Degrading Microorganisms
The isolation of CPF-degrading microbes is usually achieved through sampling from polluted agricultural soils and subsequent enrichment and screening methods. Enrichment cultures are established in minimal salt media with CPF as the only carbon or phosphorus source to enrich for organisms that can utilize the compound.[17,18]

Suman *et al.*, isolated *Staphylococcus aureus* from CPF-contaminated soil by using CPF-supplemented mineral salts medium successfully.[4] Likewise Aswathi *et al.*, isolated *Pseudomonas nitroreducens* AR-3 with CPF degradation capability at a fast rate.[19] Other significant isolations are:

- *Pseudomonas stutzeri* from pesticide-contaminated soil[20]
- *Bacillus cereus* MCAS02 from Indian crop fields[21]
- *Alcaligenesfaecalis* from oil-contaminated areas[15]
- *Bacillus* sp. H27 was isolated and identified as highly efficient CPF degrader[13]

A few researchers used metagenomic techniques to detect CPF degradation-related functional genes without needing to isolate pure cultures.[22] Cultivation-independent this technique provides further insight into contaminated soil complex microbiomes.

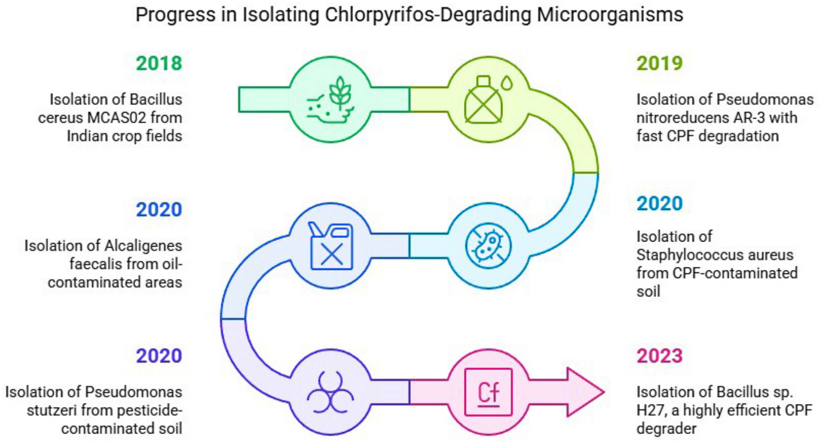

Fig. 3: Representing the Progress in Isolating Chlorpyrifos-Degrading Microorganisms.

Characterization of Isolated Microbes
After microbial strains that can biodegrade chlorpyrifos (CPF) have been isolated from farmland soils, it is necessary to comprehensively characterize them to identify their taxonomic position and determine their potential for biodegradation. Characterization generally involves phenotypic, biochemical, and molecular tests.

Phenotypic and Biochemical Tests
Phenotypic characterization is the examination of colony morphology, cell shape, motility, and Gram staining, yielding preliminary insight into microbial identity. Biochemical assays also test for metabolic functions, such as catalase and oxidase activity, nitrate reduction, and carbohydrate fermentation. Direct applicability to CPF degradation are enzymatic assays for esterase and phosphatase, given that these enzymes are involved in the hydrolysis of CPF to its intermediate 3,5,6-trichloro-2-pyridinol (TCP).[23,24] Studies indicated high esterase activity in CPF-degrading *Pseudomonas* and *Bacillus* strains, validating their involvement in primary pesticide degradation.

Molecular Identification
For precise identification, 16S rRNA gene sequencing is used in most studies. This technique enables accurate identification of isolates to the species level. Suman *et al.*, used 16S rRNA sequencing and sequence alignment software to identify their CPF-degrading isolate as *Staphylococcus* aureus.[4] Likewise Laing *et al.*, employed PCR amplification and sequencing to identify CPF-degrading isolates as *Pseudomonas*, *Bacillus*, and *Rhodococcus* genera.[25]

Whole Genome Sequencing and Genomic Insights

Genomic advances have made it possible to carry out extensive studies of microbial CPF degradation pathways. Liu performed whole-genome sequencing of *Bacillus* sp. H27, which uncovered multiple gene clusters related to CPF hydrolysis and TCP degradation, such as the opd (organophosphorus hydrolase), arylest (arylesterase), and tcpA genes. These genes encode enzymatic pathways responsible for detoxifying CPF and mineralizing TCP, providing information on the molecular machinery involved in *bioremediation.* These genomic studies give promising targets for genetic engineering and synthetic biology strategies to improve biodegradation efficiency in polluted environments.[13]

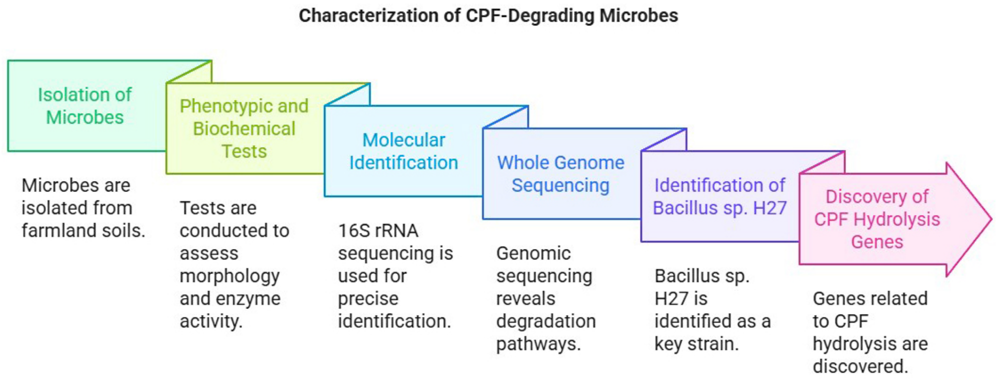

Fig. 4: Representing flowchart for Characterization of CPF-Degrading Microbes.

Degradation Pathways and Metabolites

Microbial breakdown of chlorpyrifos (CPF) comprises a sequence of biochemical processes converting the parent compound into less harmful or non-harmful metabolites. The biodegradation typically takes place through two main mechanisms

- Enzymatic hydrolysis of CPF to 3,5,6-trichloro-2-pyridinol (TCP) and diethyl thiophosphoric acid (DETP), and
- Secondary degradation and mineralization of TCP to innocuous end-products like carbon dioxide and water.

The first step in CPF degradation involves hydrolytic cleavage of the phosphorus–oxygen (P–O–aryl) bond. This key reaction is catalyzed by particular enzymes such as organophosphorus hydrolases (OPHs), arylesterases, and monooxygenases that are secreted by some bacteria in the soil. These enzymes transform CPF to intermediate metabolites like TCP and DETP, both of which are much less toxic than the parent compound.

Ambreen, isolated *Bacillus thuringiensis* MB497, which showed the ability to degrade CPF and its major metabolite TCP, thereby resulting in complete mineralization.[26] Likewise, *Pseudomonas nitroreducens* AR-3, characterized by the ability to degrade CPF rapidly and attained complete breakdown of TCP within 48 hours under favorable lab conditions, which indicates its effectiveness for efficient soil *bioremediation.*[27]

Characterized intermediate and terminal metabolites from several studies are

- TCP
- DETP
- 3,6-dichloro-2-pyridinol
- Carbon dioxide (CO_2) – indicative of complete mineralization

To explain the degradation pathway and validate the transformation of CPF and its intermediates, analytical methods like gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) are usually utilized. These instruments have been used to trace the entire process of degradation, as exemplified in the genomic and metabolomic research.[13,2] This mechanistic information is crucial in creating effective bio-based CPF remediation technologies.

Environmental and Operational Factors Affecting Biodegradation

Soil microorganism-induced efficiency of chlorpyrifos (CPF) biodegradation depends considerably on several environmental and operating factors. Such factors can act either to stimulate or suppress microbial growth and corresponding enzymatic processes responsible for pesticide degradation.

Soil pH and Temperature

Soil pH is essential for microbial metabolism and stability of enzymes. Degradation of CPF is usually favored in slightly alkaline to neutral soils (pH 6.5–8.0) because most CPF-degrading bacteria show maximum enzymatic activity under such conditions. It has been noted maximum CPF degradation by *Bacillus* and *Pseudomonas* species under such pH conditions.[7,20] Likewise, microbial growth rates and enzyme functions are influenced by temperature. The best temperature for CPF-degrading microorganisms is usually between 25°C and 35°C, above which degradation efficiency is reduced due to enzyme denaturation or microbial stress.

Moisture Content and Organic Matter

Sufficient moisture is required to sustain microbial activity and solubilize CPF, thus enhancing its bioavailability. More CPF degradation has been recorded when moist conditions were applied.[28,29] Additionally, organic amendments such as compost, manure, and biochar are not only nutrient supplies but also microbial diversity and biomass promoters, thus speeding up biodegradation processes.[17]

Pesticide Concentration

While low to moderate CPF levels will induce microbial breakdown, very high concentrations may prove toxic to the microflora in soil by inhibiting enzyme activity. Still, certain heavy-duty strains like *Bacillus* cereus and *Pseudomonas* putida were resistant to very high CPF concentrations and such strains can be used in highly contaminated environments.[21,12]

Presence of Co-contaminants

Other pesticides or contaminants may affect CPF degradation by competitive inhibition or co-metabolism. Changed degradation kinetics has been illustrated in the presence of co-contaminants, highlighting the increased complexity of natural bioremediation environments.[29,16]

Immobilization and Bioaugmentation

Operational tactics including microbial immobilization onto carriers such as alginate beads improve microbial stability and efficiency in degradation against environmental stressors. It has been demonstrated that immobilized *Bacillus* sp. H27 greatly increased CPF degradation in contaminated soils.[13] Likewise, bioaugmentation—introducing microbial consortia to contaminated environments—has been shown to effectively boost rates of degradation in situ.[30,31]

Recent Advances and Genomic Insights

Recent developments in environmental biotechnology and microbial genomics have accelerated the area of pesticide *bioremediation.* Metagenomics and high-throughput sequencing are also being used more frequently to detect new CPF-degrading microbes and enzymes, even from non-culturable populations.[22]

Whole Genome Sequencing and Functional Annotation

Whole-genome sequencing of *Bacillus* sp. H27 has been done and a series of gene clusters was found responsible for the encoding of hydrolases, mono-oxygenases, and CPF- and TCP-degradation regulatory elements.[13] Similarly metagenomics was utilized to identify novel CPF-degrading bacteria in sugarcane soils.[9]

Sophisticated tools such as KEGG pathway mapping and GO (Gene Ontology) enrichment analysis enable researchers to identify the metabolic potential, resistance factors, and survival methods of bacteria in CPF stress.[32,33]

Enzyme Engineering and Synthetic Biology

Genetic engineering strains like *Pseudomonas putida* KT2440 to produce multiple degradation genes has led to the simultaneous degradation of several pesticides, like CPF.[12] These synthetic methods increase degradation efficiency and broaden the substrate range.

Omics-Based Strategies

Transcriptomics

Facilitates understanding of gene expression following CPF exposure.[34]

Proteomics
Discloses which enzymes are engaged during various stages of degradation.

Metabolomics
Follows the metabolic fate of CPF and its intermediates.[2]

These integrative strategies enhance our knowledge of microbial physiology under the stress of pesticides and facilitate the establishment of bio-based solutions to soil detoxification.

Bioremediation Strategies and Field Applications

Although laboratory-based experiments on chlorpyrifos (CPF) degradation offer valuable information on microbial metabolism and enzyme action under controlled laboratory conditions, practical field applications offer more challenges arising from environmental heterogeneity, dynamic abiotic variability, and competition within indigenous microbial populations. In order to cope with these complications, various bioremediation approaches have been established and applied, varying from microbial inoculation to plant-microbe symbiosis.

Bioaugmentation
Bioaugmentation is the intentional introduction of particular CPF-degrading microbial strains into contaminated soil to speed up pesticide degradation. There are successes with *Alcaligenesfaecalis*, which degrades CPF but also enhances plant growth through improved nutrient uptake and stress resistance.[15] Mixed consortia of microorganisms, commonly consisting of *Bacillus*, *Pseudomonas*, and Enterobacter strains, have shown synergistic interactions resulting in more complete and effective CPF degradation.[16,35]

Biostimulation
Biostimulation involves the incorporation of organic substrates or nutrients to activate the activity of the native microbial populations. Compost, molasses, and fruit-vegetable waste are some examples of organic amendments that can act both as carbon sources and as microbial growth stimulants. Increased degradation of CPF with fruit-vegetable waste biomasshas been reported, which had enriched the metabolic capacity as well as microbial diversity of the soil.[30]

Rhizoremediation
This plant-based approach takes advantage of the symbiotic relationships between plant roots and rhizosphere soil microbes. Plants such as ryegrass, when paired with CPF-degrading bacteria such as *Bacillus pumilus*, greatly increase pesticide degradation rates while at the same time enhancing soil structure and fertility.[14,36]

Immobilized Cell Technology
For the purpose of enhancing microbial survival and activity under oscillating environmental conditions, immobilization methods have been used. Encapsulated bacteria using materials such as alginate beads, zeolite, or biochar show increased resistance and activity over extended periods. *Bacillus* sp. H27 immobilized in bio-carriers showed much higher degradation of CPF compared to free cells.[13,37]

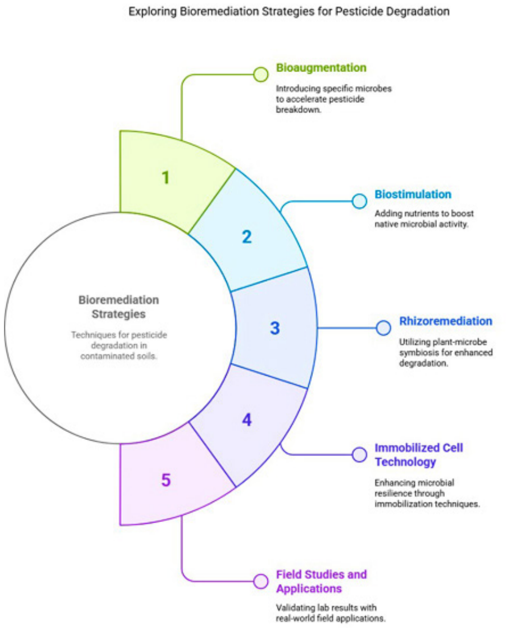

Fig. 5: Representing various bioremediation strategies for pesticide degradation.

Field Studies and Applications
Field experiments offer necessary confirmation for laboratory results. In Egyptian farmland soils, significant CPF degradation was reported within 21 days with indigenous microbial isolates.[38] Likewise, *Pseudomonas* and *Bacillus* strains were effectively

utilized in Sudanese soil to degrade CPF, malathion, and dimethoate under field conditions.[25] These field implementations affirm the viability of microbial bioremediation to curtail pesticide contamination in agricultural environments.

Challenges and Future Perspectives

Despite the potential of microbial remediation, several challenges hinder its large-scale application

Challenges

Strain Stability and Survival

Entering strains may not establish owing to predation or competition.

Environmental Variability

Soil quality, water content, pH, and temperature variations can affect degradation kinetics.

Incomplete Mineralization

TCP tends to accumulate owing to inhibited microbial capabilities in certain systems.

Regulatory Barriers

Field application of genetically modified organisms (GMOs) is controlled in most nations.

Scale-Up Challenges

Moving from lab-scale to field-scale bioremediation requires cautious optimization of formulation, delivery mechanisms, and carriers.

Future Perspectives

Microbial Consortia Design

Design of microbial consortia with complementary degradation routes may improve CPF mineralization.

CRISPR and Genetic Engineering

CRISPR and genetic engineering can design effective, multi-functional strains for site-specific treatment.

Nano-Biotechnology

Integration of nanoparticles with microbial enzymes can enhance CPF bioavailability and degradation rates under new technology.

AI-Driven Predictive Modeling

Machine learning can streamline microbial selection and forecast biodegradation kinetics under changing conditions.

Eco-toxicological Evaluations

Preventing the degradation from forming new, poisonous by-products is vital for sustainable use.[39,40]

Microbiology, environmental science, systems biology, and soil ecology must all be approached as a whole for effective, sustainable CPF bioremediation tactics to work.

Conclusion

The microbiological degradation of chlorpyrifos presents an environmentally sound, cost-efficient, and cost-effective alternative to pesticide contamination in farm soils. Many bacterial genera such as *Pseudomonas*, *Bacillus*, *Staphylococcus*, and *Alcaligenes* have shown very strong CPF-degrading potential. Application of enrichment culture methods in addition to state-of-the-art genomic and proteomic technologies has promoted a deeper knowledge of mechanisms and pathways of degradation.

While there have been successes with some laboratory-scale and field trials, areas of difficulty still include strain survival, environmental fluctuation, and partial mineralization. However, new developments in microbial consortia engineering, immobilization, and rhizoremediation show potential avenues forward. Future investigations should aim at bringing together multi-disciplinary techniques to maximize bioremediation under practical field conditions and confirm ecological safety.

With ongoing research, policy endorsement, and stakeholder participation, microbial bioremediation can become a common technique in sustainable agriculture and environmental conservation.

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Ethics Statement
This research does not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement
This study is a review of previously published literature and does not involve any new studies with human participants or animals performed by the authors. Therefore, informed consent was not required.

Permission to Reproduce Material from other Sources
Not applicable.

Author Contributions
- **Jasmeet Kaur Sohal:** designed the work and done proof reading.
- **Khushboo Minj:** searched the literature and wrote the manuscript.

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