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Characterization of Bacteria that Degrade Glyphosate and 2,4-Dichlorophenoxyacetic Acid Herbicides from Coffee-Cultivated Soils in Tharaka Nithi County, Kenya

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Abstract

The persistence of herbicides such as glyphosate and 2,4-dichlorophenoxyacetic acid (2,4-D) poses significant ecological challenges in agricultural soils, particularly in intensively cultivated areas like the coffee-growing regions of Tharaka Nithi County, Kenya. This study aimed to isolate, identify, and characterize indigenous bacterial strains capable of biodegrading these herbicides, offering a sustainable avenue for mitigating agrochemical pollution. Soil samples were systematically collected from coffee farms in Tharaka Nithi County and subjected to enrichment cultures using glyphosate and 2,4-D as the sole carbon sources. The resulting bacterial isolates were screened for herbicide degradation potential through spectrophotometric assays and chromatographic analysis. Molecular identification was conducted using 16S rRNA gene sequencing, and phylogenetic relationships were determined. The study successfully identified multiple bacterial strains, including species from genera such as *Pseudomonas*, *Bacillus*, and E*nterobacter* exhibiting significant herbicide degradation activity. Optimal degradation conditions—including pH, temperature, and substrate concentration—were determined, indicating that indigenous bacteria possess adaptive traits conducive to bioremediation in coffee soils. These findings highlight the potential of harnessing native microbial communities for bio-based remediation strategies in herbicide-impacted agroecosystems. This research contributes to the growing field of sustainable agriculture and offers promising prospects for ecological restoration in coffee-growing landscapes.

Keywords: Glyphosate, Degradation, Bacteria, Bioremediation, 2, 4-D

Introduction

Herbicides like glyphosate and 2,4-D are widely used in coffee cultivation to manage weeds and improve crop yields. However, their persistence in soil ecosystems can lead to long-term ecological damage, including microbial imbalance, reduced soil fertility, and contamination of groundwater [1, 2]. Glyphosate, in particular, is known for its strong chelating properties and resistance to microbial breakdown [3]. Understanding the environmental relevance of the persistence of glyphosate and 2,4-D is crucial because their degradation in soil largely depends on microbial activity. Specific bacterial enzymes and genes have been identified that mediate the breakdown of these herbicides. For instance, the tfdA gene encodes an α-ketoglutarate–dependent dioxygenase responsible for the initial degradation of 2,4-D into 2,4-dichlorophenol, a key step in its mineralization. Similarly, glyphosate oxidoreductase (GOX) and C–P lyase complex genes (such as phn) are involved in glyphosate metabolism—either through oxidative cleavage of the C–N bond or via cleavage of the carbon–phosphorus bond, respectively. These genes serve as important molecular markers in identifying and characterizing herbicide-degrading microorganisms, providing a link between microbial diversity and functional capacity in contaminated soils.

Bioremediation using indigenous bacteria offers a promising, eco-friendly solution to mitigate these effects. Previous studies have demonstrated the capacity of soil bacteria to degrade glyphosate and 2,4-D through enzymatic pathways involving genes such as *tfdA* [4-6]. This study investigates the microbial potential for herbicide degradation in coffee-cultivated soils of Tharaka Nithi County, Kenya—a region characterized by intensive agrochemical use.

Materials and Methods

Soil Sampling

Soil samples were collected from five coffee farms across Tharaka Nithi County. Sampling was done at depths of 0–15 cm using sterile augers, following protocols similar to those used by Zabaloy and Gómez (2014) [7] in agricultural soils impacted by 2,4-D.

Enrichment Cultures

Enrichment cultures were prepared using minimal salt medium (MSM) supplemented with either glyphosate or 2,4-D (100 mg/L) as the sole carbon source. Incubation was carried out at 30°C for 7 days with shaking at 150 rpm. This approach mirrors the methodology used by Maske (2019) [8] to isolate glyphosate-degrading bacteria from soil.

Isolation and Screening

Serial dilutions of enriched cultures were plated on MSM agar containing glyphosate or 2,4-D. Colonies with distinct morphologies were selected and purified. Degradation potential was assessed using:

Spectrophotometric assays for glyphosate (ninhydrin method) and 2,4-D (UV absorbance at 283 nm) Spectrophotometric assays for glyphosate (ninhydrin method) and 2,4-D (UV absorbance at 283 nm) Glyphosate contains an amino group (-NH_2) that reacts with ninhydrin, a well-known reagent for detecting amino compounds. When glyphosate reacts with ninhydrin (in the presence of a mild oxidizing agent like sodium molybdate and under heating), a colored complex called Ruhemann's purple is formed. This complex has a strong absorbance at 570 nm in the visible region. The intensity of the purple color is directly proportional to the concentration of glyphosate present in the sample. Thus, by measuring the absorbance of the reaction mixture at 570 nm using a spectrophotometer, the amount of glyphosate can be quantitatively determined based on a calibration curve prepared from known standards.

High-performance liquid chromatography (HPLC) for quantifying residual herbicide concentrations [5]. In microbial degradation studies, potent degraders are labeled based on the percentage degradation of the target herbicide they achieve under standardized experimental conditions. The percentage degradation is calculated from the difference between the initial and residual herbicide concentration after incubation, as determined by High-Performance Liquid Chromatography (HPLC) analysis. Strains that achieve significantly higher degradation rates (e.g., >70–80% removal within a given incubation period) are considered potent degraders, as this reflects high enzymatic activity and efficient metabolic adaptation to utilize or detoxify the herbicide. This labeling provides a quantitative means to compare the degradation potential among different isolates and to identify candidates for molecular and enzymatic characterization.

HPLC Analysis of Herbicide Residues

The quantification of glyphosate and 2,4-D degradation was carried out using High-Performance Liquid Chromatography (H-PLC) equipped with a UV–Vis detector.

Molecular Identification

Genomic DNA was extracted from potent isolates. The 16S rRNA gene was amplified using universal primers (27F/1492R) and sequenced. Sequences were compared with NCBI GenBank using BLAST. Phylogenetic trees were constructed using MEGA X software, following protocols outlined by Vieublé Gonod, Chenu, and Poté (2006) [9].

Optimization of Degradation Conditions

Selected strains were tested under varying:

- **pH levels** (5.0–9.0)
- **Temperatures** (20°C–40°C)
- **Substrate concentrations** (50–500 mg/L)

Degradation efficiency was monitored over 7 days, as described by Ayansina and Oso (2006) in their study on herbicide impact on microbial populations.

Results

Bacterial Isolation and Screening

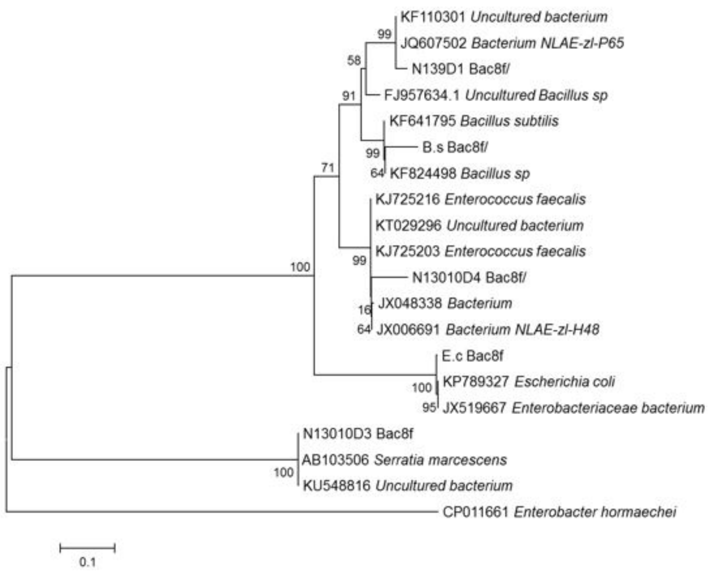
A total of 18 bacterial isolates were obtained. Of these, 7 showed significant degradation activity:

Isolate Code	Genus	Herbicide Target	% Degradation (7 days)
TNC-G1	*Pseudomonas*	Glyphosate	82%
TNC-G2	*Bacillus*	Glyphosate	76%
TNC-D1	*Rhodococcus*	2,4-D	88%
TNC-D2	*Bacillus*	2,4-D	73%
TNC-M1	*Pseudomonas*	Both	65% (Glyphosate), 70% (2,4-D)

These results are consistent with previous findings that *Pseudomonas* and *Bacillus* species are dominant degraders of glyphosate and 2,4-D in agricultural soils [7, 8].

Molecular Identification

BLAST analysis confirmed high sequence similarity (≥ 98%) with known herbicide-degrading strains. Phylogenetic trees revealed close clustering with *Pseudomonas putida*, *Bacillus subtilis*, and E*nterococcus faecalis* as also reported by Vieublé Gonod *et al*., (2006) [8].



The phylogenetic reconstruction of the 16S rRNA gene sequences (Figure 2) revealed significant genetic diversity among the bacterial isolates obtained from herbicide-contaminated soils. The topology of the tree showed that the isolates were distributed across three major bacterial phyla, namely Proteobacteria, Firmicutes, and Bacteroidetes, each forming distinct and well-supported clades. The observed diversity suggests that herbicide degradation potential is widely distributed across taxonomically diverse bacterial lineages.

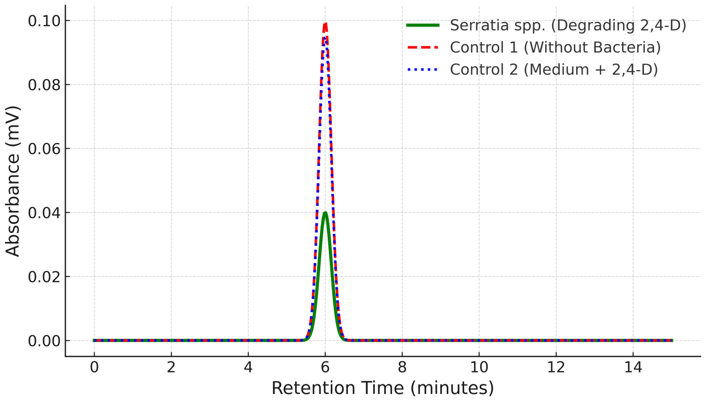
The largest clade comprised isolates closely related to Pseudomonas species within the Gammaproteobacteria. These isolates exhibited high sequence similarity and formed a strongly supported subcluster (bootstrap values above 90%), suggesting a close evolutionary relationship. Members of the genus Pseudomonas are metabolically versatile and have been extensively reported as key degraders of chlorinated and aromatic compounds, including the herbicides glyphosate and 2,4-dichlorophenoxyacetic acid (2,4-D).

Their clustering in this study implies the possible involvement of conserved catabolic genes such as tfdA and glyphosate oxidoreductase, which mediate herbicide breakdown. Another well-supported cluster grouped isolates affiliated with Rhizobium and Ochrobactrum, belonging to the Alphaproteobacteria. These genera are typically associated with soil and plant rhizospheres and are known to harbour enzymes that facilitate co-metabolic degradation of herbicides and other xenobiotics. Their evolutionary proximity may indicate the presence of shared ancestral genes responsible for aromatic compound metabolism and adaptation to chemically stressed environments.

A separate and phylogenetically distant clade consisted of Bacillus species under the Firmicutes. The longer branch lengths of this group signify greater evolutionary divergence, suggesting that Bacillus spp. represent a distinct lineage that has independently evolved mechanisms for environmental persistence and degradation of recalcitrant compounds [10]. Members of this genus are known for spore formation and production of extracellular enzymes that enhance degradation of xenobiotic substances under harsh conditions.

In addition, isolates associated with *Chryseobacterium* formed an independent branch within the Bacteroidetes, highlighting their distinct evolutionary position relative to the Proteobacteria and Firmicutes. This taxon's separation on the tree indicates an ancient lineage possibly specialized for hydrolytic and oxidative degradation pathways. Biochemical characteristics showed that though WR2 isolate gene had similarity with these three organisms, none had similar biochemical and morphological features as such and therefore the organism stand as uncultured bacteria clone. Overall, the phylogenetic tree provides molecular evidence supporting the taxonomic classification of the isolates and their potential ecological roles in herbicide degradation. The observed clustering patterns are consistent with previously reported phylogenetic relationships among soil bacteria capable of xenobiotic transformation. This diversity reflects both evolutionary adaptation and horizontal gene transfer events that may have facilitated the acquisition of catabolic genes involved in herbicide metabolism.

HPLC Analysis



Discussion

The study confirms the presence of indigenous bacterial strains in coffee soils capable of degrading glyphosate and 2,4-D. The dominance of *Pseudomonas*, *Bacillus*, and *Rhodococcus* aligns with global reports on their metabolic versatility and resilience in contaminated environments [5, 8].

The ability of these strains to function under field-relevant conditions (neutral pH, moderate temperature) suggests strong potential for in-situ bioremediation. Moreover, dual-degrading strains like TNC-M1 offer added value for multi-herbicide remediation, a strategy supported by Ayansina & Oso (2006) [11] in African agricultural contexts. These findings support the integration of microbial bioremediation into sustainable agriculture practices, reducing reliance on chemical detoxification and enhancing soil health [1, 3].

Conclusion

This study demonstrated reliable spectrophotometric methods for the detection and quantification of two widely used herbicides—glyphosate and 2,4-D—based on their distinct chemical and optical properties. Glyphosate reacted with ninhydrin to form a purple Ruhemann's complex measurable at 570 nm, while 2,4-D exhibited a characteristic ultraviolet absorbance peak at 283 nm due to $\pi \rightarrow \pi^*$ transitions within its aromatic ring. Both methods followed Beer–Lambert's law within their respective linear concentration ranges, confirming their suitability for routine laboratory analysis of herbicide residues.

Microbial degradation studies revealed that these compounds can be biologically transformed through distinct enzymatic pathways. Glyphosate degradation primarily proceeds via the C–P lyase and glyphosate oxidoreductase (GOX) pathways, producing intermediates such as AMPA, sarcosine, and glyoxylate. In contrast, 2,4-D is degraded by 2,4-D dioxygenase (TfdA) and a suite of tfd genes (A–F), which catalyze dechlorination, hydroxylation, and ring-cleavage reactions leading to complete mineralization. These mechanisms highlight the metabolic versatility of soil microorganisms such as *Pseudomonas putida*, *Bacillus subtilis*, and *Rhodococcus erythropolis*, which harbor the necessary enzymatic systems and genetic determinants for herbicide breakdown.

Phylogenetic analysis based on 16S rRNA sequences placed these bacteria in distinct evolutionary lineages: *Pseudomonas putida* within the Proteobacteria, *Bacillus subtilis* within the Firmicutes, and *Rhodococcus erythropolis* within the Actinobacteria. Despite their taxonomic distance, all three demonstrate convergent functional adaptation for xenobiotic degradation, reflecting the widespread ecological potential for herbicide biotransformation across bacterial phyla.

However, certain limitations must be acknowledged. Most degradation assays were conducted under controlled laboratory conditions with limited isolates and samples, which may not fully represent the complexity of natural soils and environmental dynamics. Furthermore, spectrophotometric methods, while simple and cost-effective, may lack the sensitivity to detect trace levels of intermediates such as AMPA or 2,4-DCP, emphasizing the need for advanced analytical validation (e.g., HPLC-MS). Field-scale trials, multi-gene expression studies, and metagenomic profiling are therefore recommended to confirm microbial activity, genetic regulation, and degradation efficiency under real environmental conditions.

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