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Land degradation

Characterization of a *Pseudomonas* sp. Isolated from Langkawi Capable of Degrading Glyphosate

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ABSTRACT

Bioremediation of soil contaminants, including glyphosate, is an economically viable and environmentally friendly technique. Glyphosate, one of the most widely used herbicides for weed management, poses significant risks to wildlife and their habitats when it contaminates the environment. This study focused on the bioremediation potential of soil *Pseudomonas* spp. isolated from a paddy field with a long history of glyphosate application. The most promising isolate was tentatively identified as *Pseudomonas* sp. strain UPM-2009 through partial identification methods. This bacterium showed significant potential for glyphosate degradation under optimal conditions. Experiments demonstrated that *Pseudomonas* sp. degrades glyphosate most effectively at pH 7.0, a glyphosate concentration of 0.5 g/L, temperatures between 30 and 35°C, and an inoculum size of 1% (v/v). Notably, the bacterium exhibited a two-day lag period at 0.5 g/L glyphosate, achieving nearly 90% degradation after six days of incubation. Heavy metals such as Hg(II), Ag(I), and Cd(II) significantly inhibited bacterial growth, with inhibition rates of 99%, 95%, and 66%, respectively. This study underscores the potential of *Pseudomonas* sp. for bioremediation of glyphosate-contaminated environments. It highlights the need for further research, particularly molecular identification techniques, to fully characterize and optimize this bioremediation strategy. This approach can significantly contribute to mitigating the environmental impact of glyphosate pollution, promoting healthier ecosystems and sustainable agricultural practices.

INTRODUCTION

Pollution levels in the environment are alarmingly high across the globe. Urbanization, industrialization, and general economic expansion have all contributed to rising energy use and trash output. Additional research is needed to fully understand the global health risks posed by various environmental degradations, such as acid deposition, water pollution, improper waste disposal, and greenhouse gas emissions. This research should draw from a range of academic disciplines and research methods, including public health, public policy, environmental engineering, and public health promotion and disease prevention [1–4]. Multiple adverse health effects, such as prematurity, respiratory illnesses, allergies, cancers, heart disease, elevated oxidative stress, endothelial dysfunction, mental health issues, and many more, can be caused by early exposure to environmental contaminants. Multiple studies have linked environmental particulate matter exposure to an increased risk of health problems, including

cancer, organ damage, and other long-term illnesses and even death [5–10]. As a systemic herbicide, glyphosate does not exhibit any selectivity. It is used to manage the majority of annual and perennial plants. The existence of glyphosate metabolites in these products is causing increasing alarm over public safety. Despite animal studies showing that high dosages of glyphosate were toxic to organs, reproduction, and the neurological system, there is little evidence that human exposure to the herbicide is carcinogenic. Due to its extensive usage in agriculture, glyphosate has been detected in several environmental samples, including water. Questions of how to prevent glyphosate accumulation (in soils and water sources) and how to eliminate it once it has occurred are receiving more and more attention as evidence mounts that glyphosate is harmful to living beings [11–18].

The world's herbicide use is expected to be between 125,000 and 130,000 metric tons per year. Concerns about the toxicity and possible health effects of glyphosate have led to its restriction from agricultural usage in over 20 nations. Many farmers in countries like Malaysia disregard these laws and use glyphosate anyhow, often without the necessary protective gear, putting their health at serious risk. A growing number of experts are recommending glyphosate bioremediation over more conventional physical and chemical degradation techniques in light of these concerns. Bioremediation is an eco-friendly and maybe safer way to handle glyphosate pollution in agricultural settings; it uses microbes' inherent metabolic activities to degrade glyphosate into less dangerous compounds [19–22].

In archaea, bacteria, Apicomplexa, algae, fungi, and plants, glyphosate inhibits the enzyme 5-enolpyruvyl-shikimate-3-phosphate (EPSP) synthase, which is a key component of the shikimate pathway. This pathway is crucial for the de novo synthesis of aromatic amino acids such as phenylalanine, tyrosine, and tryptophan, as well as the vitamins folic acid and menaquinone. EPSP synthase catalyzes the conversion of shikimic acid-3-phosphate (S3P) and the glycolytic intermediate phosphoenolpyruvate (PEP) into EPSP. By suppressing EPSP synthase, glyphosate effectively disrupts the production of these essential compounds, leading to the inhibition of growth and development in these organisms [23–26]. Bacteria that can utilize glyphosate are excellent candidates for the bioremediation of glyphosate in the Malaysian environment. Therefore, this study aims to screen for such bacteria from a paddy field with a long history of glyphosate applications. By isolating and identifying bacteria capable of degrading glyphosate, this research seeks to develop effective bioremediation strategies to mitigate glyphosate contamination in agricultural settings.

MATERIALS AND METHODS

Chemicals

All chemicals used in this work were of analytical grade. Glyphosate (N-(phosphonomethyl)glycine) was purchased as a technical grade chemical (95%, Zhengzhou Delong Chemical Co., Ltd . Media preparation was based on the recipe [9] except otherwise stated here. All the experiments involving microorganisms were done in a class II biosafety cabinet.

Screening of glyphosate-degrading isolate and growth medium

Soil was sampled from a paddy field in Langkawi, Kedah. Soil was sampled 5 cm from the topsoil using a sterile spatula and placed in a sterile polycarbonate container. Soil sample (1 g) was added to 9 mL of sterile tap water and mixed. Then, 0.2 mL aliquot was transferred and streaked on a mineral salt agar medium (pH 7.5) with the following composition in g/L; 0.5 g of NaCl, 0.5 g of KCl, 2 g of NH_4SO_4, 0.2 g of $MgSO_4.7H_2O$, 0.01 g of $CaCl_2$, 0.001 g of $FeSO_4$, 18 g of agar (solidifying agent). A stock solution of glyphosate (10 g/L) was prepared in deionized water, and the final concentration of glyphosate used as the only phosphate source was 1 g/L. Glyphosate's maximum solubility in deionized water was about 12 g/L. The medium was then autoclaved at 121 ºC, 115 kPa for 15 min, and glyphosate was added to the medium through filter sterilization (0.2 micron filter). Two distinct colonies formed, indicating the presence of glyphosate-degrading microorganisms. These colonies were restreaked on fresh agar medium. A single colony was transferred into 10 mL of glyphosate MS medium in 28 mL-universal bottles and incubated at room temperature for 3 d on an orbital shaker at 150 rpm.

The best glyphosate-degrading bacterium based on A600 nm measurement was utilized for further optimization. The *Pseudomonas* bacterial cultures long-term storage is adopted from [27]. Briefly, an overnight culture was grown in LB broth at 30 °C with shaking at 150 rpm until the exponential growth phase, confirmed by an optical density (A600 nm) of approximately 0.6-0.8. A 50% glycerol solution was prepared by diluting sterile glycerol with sterile water and autoclaved at 121°C for 15 minutes. Sterile cryovials were labeled accordingly and aliquoted with 500 µL of the 50% glycerol solution. Afterwards, 500 µL of the *Pseudomonas* culture was added to each cryovial, yielding a final glycerol concentration of 25%. The mixture was vortexed briefly to ensure uniform distribution of the cells in the glycerol solution. Finally, the cryovials were immediately transferred to a -80°C freezer for long-term storage.

Characterization of glyphosate degradation

The experiments were conducted using a microplate titer method. The glyphosate-MSM medium mentioned earlier was transferred into the microplate using a pipette, with 200 µL of the medium being added. Additionally, 20 µL of bacteria inoculum was mixed into the wells of the microplate. Subsequently, the microplate was placed under a cover and kept in a stationary position for a duration of 5 days at ambient temperature. The growth of the bacterial isolate during glyphosate degradation was evaluated by examining the impact of many elements individually, including pH, concentration, temperature, heavy metals, inoculum size, and aeration. This approach, known as one factor at a time (OFAT), allowed for a comprehensive understanding of how each factor influences the growth process. The characterisation of the optimal isolate involves investigations into the impact of incubation duration, glyphosate concentration, inoculum size, heavy metal impacts, pH, and temperature.

Determination of glyphosate using HPLC

Glyphosate degradation was monitored using an HPLC [28]. An isocratic gradient elution method was employed in this study. The instrumentation included an Agilent 1200 series equipped with an autosampler and a UV detector. Chromatographic separation was achieved using a Zorbax Agilent SAX column with dimensions of 4.6 mm ID x 250 mm and a particle size of 5 µm. The mobile phase comprised 6.2 mM KH2PO4 in 4% (v/v) methanol, with the pH adjusted to 2.1 using 85% phosphoric acid. The flow rate was maintained at 1 mL/min, and the detection wavelength was set at 195 nm.

Morphological, physiological, and biochemical characterization

Using conventional morphological and biochemical techniques as outlined in Bergey's Manual of Determinative Bacteriology, the bacterium was biochemically and phenotypically described [29]. Interpretation of the results was carried out via the ABIS online system [30].

Statistical analysis

All experiments were conducted in triplicate to ensure the reliability and reproducibility of the results. Experimental errors were represented as standard deviation bars. Data were statistically analyzed using GraphPad Instat software. Statistical significance was determined using one-way ANOVA with a 95% confidence interval.

RESULTS AND DISCUSSION

Screening of the Isolates

The screening yielded two distinct colonies of bacterium able to utilize glyphosate as a phosphorous source. The best isolate based on a higher absorbance value at A600 nm was chosen for partial identification.

Partial identification of the bacterium

The bacterium was a Gram-negative, rod-shaped, motile microorganism. Culture, morphology, and a battery of biochemical analyses all pointed to the same bacterium, which allowed for its identification (**Table 1**) to the Bergey's Manual of Determinative Bacteriology [29] and using the ABIS online software [30]. The software gave three suggestions for the bacterial identity with the highest homology (99%) and accuracy at 88% as *Pseudomonas aeruginosa*. However, more works in the future especially molecular identification technique through comparison of the 16srRNA gene are needed to identify this species further. However, at this juncture, the bacterium is tentatively identified as *Pseudomonas* sp. strain UPM-2009.

Numerous bacteria from this genus is known for their ability to degrade pesticides including glyphosate [31–38]. Hence, at this juncture, the assignment to the species level cannot be done. More work in the future especially molecular identification technique through comparison of the 16srRNA gene are needed to identify this species further. Other glyphosate-degrading bacterium includes *Alcaligenes* sp. [31], *Flavobacterium* sp. [39], *Bacillus megaterium* [40], *Geobacillus caldoxylosilyticus* [41], Enterobacter cloacae [37], *Rhizobium* sp. and *Agrobacterium* sp. [42] and R. aquatilis [43].

Pseudomonas bacteria are well known for their skill, in breaking down substances thanks to their ability to adapt to different environments diverse genetic makeup, efficient ways of expelling toxins formation of protective biofilms and their symbiotic relationships. They have a metabolism that allows them to use organic compounds for energy with the help of a wide range of enzymes. Their large genomes contain genes that help break down substances and they can even acquire new genes through gene transfer. *Pseudomonas* bacteria can get rid of toxins efficiently using pump systems, which helps them survive in environments with levels of harmful substances. By forming biofilms that act as shields they can thrive in conditions. These bacteria also produce enzymes when exposed to substances ensuring effective breakdown.

Their ability to thrive in settings like soil, water and plants shows how well suited they are for biodegradation tasks. Moreover, *Pseudomonas* can form relationships with plants and other microorganisms that enhance their ability to break down harmful chemicals effectively. These unique traits make *Pseudomonas* highly effective in cleaning up environments by transforming chemicals into less harmful forms. Showcasing their importance, in environmental conservation efforts [44–46].

Table 1. Biochemical tests for *Pseudomonas* sp.

Motility	+	Utilization of:	
Hemolysis	+	L-Arabinose	–
Growth at 4 ºC	–	Citrate	+
Growth at 41 ºC	+	Fructose	+
Growth on MacConkey agar	+	Glucose	+
Arginine dihydrolase (ADH)	+	meso-Inositol	–
Alkaline phosphatase (PAL)	–	2-Ketogluconate	+
H_2S production	–	Mannose	–
Indole production	–	Mannitol	+
Nitrates reduction	+	Sorbitol	–
Lecithinase	–	Sucrose	–
Lysine decarboxylase (LDC)	–	Trehalose	–
Ornithine decarboxylase (ODC)	–	Xylose	–
ONPG (beta-galactosidase)	–	Starch hydrolysis	–
Esculin hydrolysis	–		
Gelatin hydrolysis	+		
Starch hydrolysis	d		
Oxidase reaction	+		

Note: + positive result, − negative result, d indeterminate result

Characterization of glyphosate degradation

Effect of initial pH

The impact on glyphosate breakdown in the pH range of 5.5–8.5 was measured. After 4 days of incubation, the results demonstrate that between pH 6.0 and pH 7.0 is optimal for bacterial growth based on ANOVA analysis, with growth significantly reduced ($p<0.05$) at values above the optimal value **(Fig. 1).**

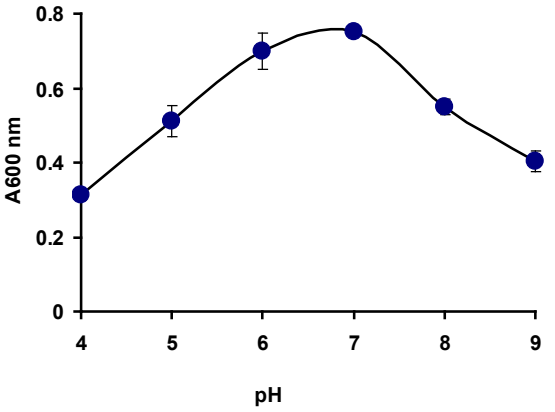

Fig 1. Effect of initial pH on glyphosate degradation by *Pseudomonas* sp. strain UPM-2009. The error bar represents the mean ± standard deviation (n=3).

Effect of glyphosate concentration on glyphosate reduction

From concentrations of 0.1 to 1 g/L, the impact of carbon source on this bacteria's ability to degrade glyphosate was measured. Results reveal that the best glyphosate concentrations to support growth were between 500 and 700 mg/L **(Fig. 2)**.

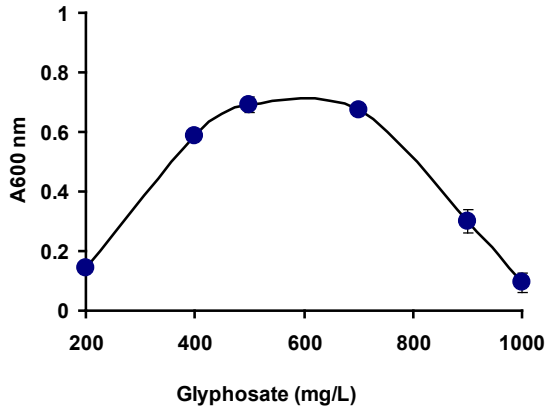

Fig 2. Effect of glyphosate concentration on glyphosate degradation by *Pseudomonas* sp. strain UPM-2009. Error bars represent mean ± standard deviation (n=3).

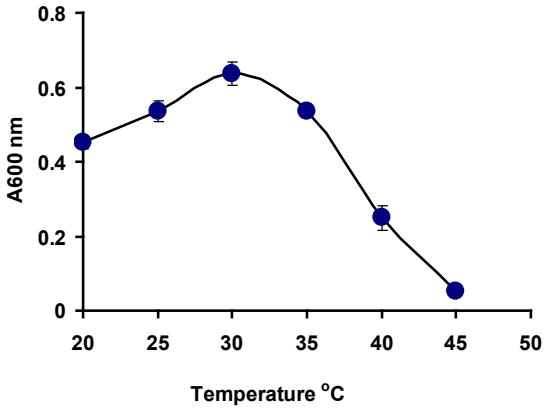

Fig 4. Effect of temperature on glyphosate degradation by *Pseudomonas* sp. strain UPM-2009. The error bar represents the mean ± standard deviation (n=3).

Effect of inoculum size on Glyphosate biodegradation

Different inoculum sizes, from 0.1 to 1 percent (v/v) from an initial stock of A600 nm of 1.0, were tested to determine their impact on glyphosate reduction. The data reveal that as the size of the inoculum was raised, the growth was also increased (**Fig. 3**).

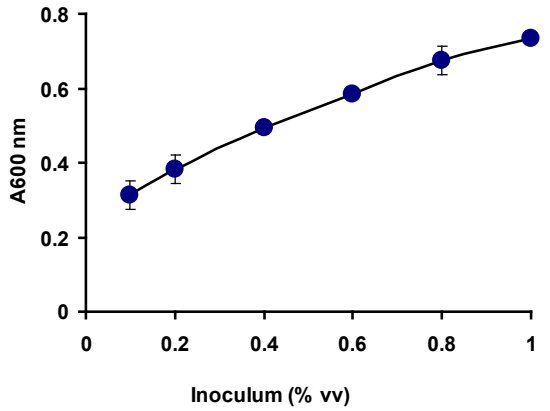

Fig 3. Effect of inoculum sizes (% v/v) on the growth of glyphosate-degrading *Pseudomonas* sp. strain UPM-2009. The error bar represents the mean ± standard deviation (n=3).

Effect of temperature

Biodegradation of glyphosate was investigated at temperatures between 25 and 50 °C. The results suggest that the optimal temperature occurred at 30 °C, with a statistically significant ($p<0.05$) decrease in growth observed at higher temperatures (**Fig. 4**).

Growth of bacterium and degradation of glyphosate

The growth of this bacterium at 500 mg/L glyphosate shows a lag period that last about a aday (**Fig. 5**). Glyphosate concentration was decreases concomitant to cellular growth. Abiotic degradation of glyphosate was minimal as judged by the control.

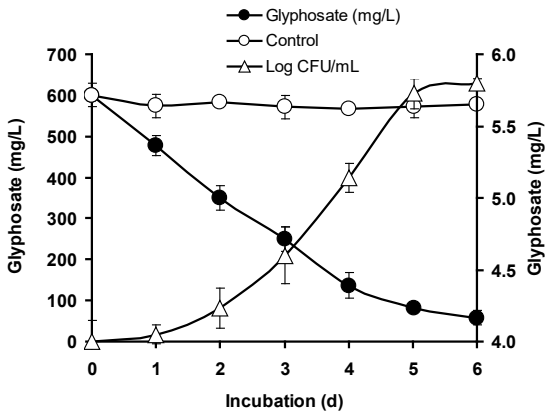

Fig. 5. Growth profile of *Pseudomonas* sp. strain UPM-2009 on 500 mg/L glyphosate. Each data point represents the mean ± standard deviation of three replicates.

The effect of heavy metals

Heavy metals such as Hg(II), Ag(I) and Cd(II) caused 99, 95 and 66% inhibition of growth on glyphosate. Other metals are considered not inhibitory (**Fig. 6**).

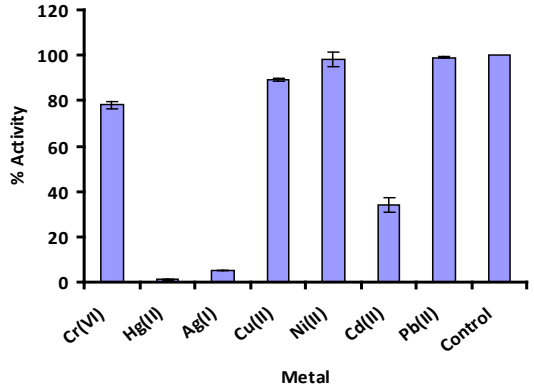

Fig. 6. Effect of 1 mg/L of heavy metals on growth of *Pseudomonas* sp. strain UPM-2009 on 500 mg/L glyphosate. Each data point represents the mean ± standard deviation of three replicates.

DISCUSSION

There may be a way to prevent the buildup of dangerous intermediates that can result from incomplete breakdown if the inserted microbial strains can fully metabolize glyphosate. To ensure that the breakdown products are non-toxic and environmentally benign, this entire metabolism is essential in bioremediation operations. In order to foster the development of microbes or to maintain circumstances favorable for a biological reaction, incubation entails keeping a certain set of environmental parameters constant. Because it creates an ideal environment for microbes to thrive, this method is fundamental in bioremediation. Ensuring that bacteria have the environmental stability they need to work efficiently is the goal of proper incubation. All living things, including microbes, rely on nutrition for survival, growth, and metabolic processes. Depending on how readily these nutrients are available, their growth and metabolism may be affected by chemicals like glyphosate.

According to the research, microbes' ability to use a low-carbon source may be inversely related to their growth potential. Because of this, microbes may be able to degrade chemicals like glyphosate more efficiently in settings where carbon sources are scarce. The possibility that additional carbon sources can accelerate glyphosate breakdown was not investigated in the study. Future studies should take this into account, since different carbon sources may affect the breakdown routes and efficiency of microbes. Nevertheless, glyphosate was most effectively degraded at doses ranging from 500 to 700 mg/L when no other carbon sources were present. Bioremediation techniques that aim to clean up glyphosate contaminations can benefit from this discovery because it shows that certain microbial strains can use glyphosate as their only carbon source. There are microorganisms that can degrade higher concentrations of glyphosate. Or instance, both *Acetobacter* sp. and *P. fluorescens* were shown to grow best at 7500 ppm (7.5 g/L), despite being able to survive concentrations of glyphosate as high as 250,000 ppm (250 g/L) [34].

Understanding the impact of inoculum size on glyphosate reduction is crucial due to the demonstrated negative correlation between high inoculum concentration and bacterium development, which therefore affects glyphosate breakdown. Requirement of bacterial isolate to grow in an optimal manner means that it is necessary to employ the correct amount of bacterial inoculum. If an insufficient amount is used, the nutrients in the medium will be exhausted prior to the bacterium having the opportunity to consume them, resulting in the failure of the experiment. Conversely, a high concentration might result in mortality and growth suppression due to the expected scarcity of nutrients in the environment, leading to insufficient nourishment and thus hindered growth [47].

The maintenance of a medium's pH is crucial because it affects cell growth and proliferation in bacterial settings. At pH values below 5, glyphosate degradation was not maintained, probably because acidic conditions are not conducive to bacterial development. Bacteria, like other microbes, demand a proper and physiological pH in order to survive and carry out their metabolic operations, and pH is a measure of the degree to which a medium is acidic, neutral, or alkaline. Because the pH scale quantifies how acidic, neutral, or alkaline a given medium is [48].

Their ability to regulate the pH gradient between their intracellular and external environments is crucial to their survival at pH values above and below 5.0 [49]. *Bacillus cereus* CB4 [50] demonstrated optimal glyphosate breakdown at pH 6.0 – 7.0, whereas *Pseudomonas putida* favored an environment with a higher alkaline concentration., with optimum growth at pH 9.0 [36]. However, there is a lack of published research on glyphosate-degrading bacteria that thrive in acidic environments. This is because the majority of glyphosate degraders require a pH range of neutral to alkaline for the best breakdown rate [39,42,51–58] including several glyphosate-degrading *Pseudomonas* spp. [26,33,36,38,40,59–62]

Several bacteria have been found to be glyphosate degraders at different temperatures; nevertheless, 30–35 °C seems to be the best spot. *Ochrobactrum* sp. GDOS [53] and *Pseudomonas putida* [36]. Glyphosate use is strongly indicated by the fact that the largest bacterial growth occurs at 30 °C. Our bacterium's high preferred temperature may have anything to do with the hot environment in which it was originally found. Mesophilic bacteria make up the majority of glyphosate degraders, and their ideal development temperature range is 25–35 °C [39,42,51–58] and also including several glyphosate-degrading *Pseudomonas* spp. [26,33,36,38,40,59–62]. *Geobacillus caldoxylosilyticus* [41] is thermophilic glyphosate-degrading bacterium which as an optimum temperature at 60 °C.

Many bacteria of the genus *Pseudomonas* have been found to digest glyphosate; therefore this study is not the first to find one. that glyphosate is most often degraded in laboratory settings by bacteria of the genus *Pseudomonas*, including multiple species of *Pseudomonas* that are capable of digesting glyphosate [26,33,36,38,40,59–62] (**Table 1**). Their resistance to the suppression of the 5-enolpyruvylshikimate-3-phosphate synthase enzyme (EPSPS) is a result of gene mutation and duplication, which allows these bacteria to grow on glyphosate [25].

Consistent with several papers showing a significant decrease in microbial population upon addition of glyphosate to the medium culture, the modest number of strains isolated from the medium containing glyphosate as the sole carbon or phosphorus source is acceptable. This finding makes sense when considering the toxicity of synthetic media as a result of glyphosate's method of action, which is to obstruct the shikimic acid pathway. When an organism is exposed to glyphosate, it loses the ability to make aromatic amino acids, which leads to cell death. This is because almost every kind of microbe has a shikimic acid pathway.

Table 1. Characterizations of several glyphosate-degrading microorganisms.

Name of Microorganisms	Optimum pH for Degradation	Optimum Temperature for Degradation	Level of Glyphosate Degraded	As Carbon or Phosphate Source	Inhibiting Heavy Metals	Ref
Burkholderia vietnamiensis	6.0	30	91 and 74% of 50 ppm	Phosphate	-	[58]
Burkholderia sp. AQ5–12	6.0	30	91 and 74% of 50 ppm	Phosphate	-	[58]
Klebsiella oxytoca strain Saw-5	7.0	30	200 mg/L	Carbon	-	[63]
Pseudomonas sp.	7.0	40	High	Carbon	Cu, Zn, Pb, Hg, Ag, Fe	[38]
Bradyrhizobium sp.	6.0-7.0	30	Moderate	Phosphate Source	-	[64]
Trichosporon cutaneum	5.5-6.0	28	58%	Carbon	-	[65]
Candida tropicalis	5.5-6.0	28	76%	Carbon	-	[65]
Aspergillus niger	3.5-4.0	25	High	Carbon	Not Specified	[66]
Fusarium oxysporum	5.0-5.5	25	High	Carbon	-	[66]
Penicillium spinulosum	4.5-5.0	27	Moderate	Carbon	-	[66]
Aspergillus terreus	4.5-5.0	27	Moderate	Carbon	-	[66]
Aspergillus flavus	4.5-5.0	27	Moderate	Carbon	-	[66]
Mucor spp.	5.0-5.5	25	Moderate	Carbon	-	[66]
Rhizopus stolonifer	5.5-6.0	28	High	Carbon	-	[66]
Trichoderma koningii	5.5-6.0	28	High	Carbon	-	[66]
Trichosporon cutaneum	5.5-6.0	28	58%	Carbon	-	[65]
Enterobacter bugandensis	7.0	30	High	Carbon	Cd, Pb	[67]
Klebsiella sp.	6.5-7.5	35	High	Carbon	Cu, Zn, Pb	[68]
Arthrobacter sp.	6.5-7.0	30	High	Carbon	Cd, Pb	[69]
Bacillus sp.	7.0-7.5	37	High	Carbon	Cu, Zn	[70]
Sphingomonas sp.	6.5-7.0	28	Moderate	Carbon	Hg, Pb	[71]
Agrobacterium sp.	6.0-6.5	30	Moderate	Carbon	Cu, Zn, Pb	[66]
Burkholderia sp.	7.0-7.5	35	High	Carbon	Cd, Zn, Pb	[72]
Streptomyces sp.	6.5-7.0	28	Moderate	Carbon	Cu, Pb	[73]
Paenibacillus sp.	7.0-7.5	30	High	Carbon	Zn, Pb	[70]
Pseudomonas putida	9.0	30	1000 mg/L	Phosphate	-	[36]
Bacillus subtilis strain Bs-15	8.0	35	10,000 mg/L	Phosphate	-	[74]
Pseudomonas sp. strain UPM-2009	7.0	30 to 35°C	500 mg/L	Phosphate	Hg, Ag and Cd	This study

Growth of *Sphingomonas* sp. [71] on glyphosate as a carbon source and *Pseudomonas* sp. as a phosphate source [38] was also inhibited by mercury indicating a strong inhibition of biodegradation by this toxic metal. Many other works on glyphosate biodegradation do not study the inhibitory effect of heavy metals which is unfortunate since many sites contaminated with organic contaminants are also co-contaminated with heavy metals especially mercury. This point is crucial as it brings attention to a gap, in research.

Many studies focusing on breakdown often overlook the hindering effects of heavy metals. This omission is especially worrisome considering that numerous sites contaminated with pollutants like glyphosate are also tainted with metals such as mercury. The simultaneous existence of these pollutants can complicate efforts to clean up the environment, underscoring the importance of understanding and addressing the impacts of metals on microbial degradation processes. Further investigation is necessary to study how heavy metals interact with degradation mechanisms in order to develop strategies for environmental cleanup. This research will help ensure that heavy metals do not hinder the breakdown of pollutants ultimately improving the effectiveness of cleanup endeavors.

CONCLUSION

Two distinct colonies were identified as capable of utilizing glyphosate as a phosphorus source during the screening of bacterial isolates from a paddy field. The most promising isolate was provisionally identified as *Pseudomonas* sp. strain UPM-2009 through partial identification methods, based on its growth characteristics. Under optimal conditions, this motile, rod-shaped, Gram-negative bacterium exhibited substantial potential for glyphosate degradation. *Pseudomonas* sp. effectively degrades glyphosate at a specific pH, glyphosate concentration, temperature range, and inoculum size, as demonstrated by the experiments. The bacterium demonstrated a significant adaptation to these conditions, demonstrating efficient degradation throughout the incubation period. Nevertheless, the bacterium's growth and degradation capabilities were substantially impaired by the presence of heavy metals. In conclusion, this investigation underscores the potential of *Pseudomonas* sp. to facilitate the bioremediation of glyphosate-contaminated environments. The results indicate that this strain has the potential to be a valuable tool in the mitigation of glyphosate pollution in agricultural environments, particularly through the application of molecular identification techniques and additional research and optimization.

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