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Characterisation of a *Bacillus* sp. Isolated from Soils Near Lake Maninjau Capable of Degrading Glyphosate

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ABSTRACT

Glyphosate and other soil toxins can be ecologically and financially sustainable bioremediation. When it pollutes the environment, the herbicide glyphosate—used extensively for weed control—poses serious dangers to animals and their ecosystems. A rice field that had been treated with glyphosate for several years was the subject of this investigation into the bioremediation capabilities of soil bacteria. We provisionally identified the most promising isolate as *Bacillus* sp. strain Unand1 using partial identification methods. Under ideal circumstances, this bacterium demonstrated considerable promise for degrading glyphosate. Bacterial experiments showed that the optimal conditions for glyphosate degradation were a pH of 6.0–7.0, a glyphosate concentration of 0.5–0.6 g/L, 30 °C, and a 1% (v/v) inoculum size. At 0.5 g/L glyphosate, the bacterium showed a two-day lag phase before degrading over 90% after six days of incubation. There was a remarkable 99% and 95% suppression rate of bacterial growth, respectively, when exposed to heavy metals such as Hg(II) and Ag(I). According to this study, this bacterium has bioremediation capability for glyphosate-contaminated settings. Full characterisation and optimization of this bioremediation approach require additional research, especially using molecular identification techniques. This method has the potential to greatly reduce the negative effects of glyphosate pollution on ecosystems and agricultural sustainability.

INTRODUCTION

Worldwide, environmental pollution levels are extremely high. Energy consumption and waste production have risen due to urbanization, industrialisation, and overall economic expansion. More studies are required to determine the exact nature of the dangers that acid deposition, water contamination, incorrect waste disposal, and greenhouse gas emissions pose to human health worldwide. Public health, environmental engineering, public policy, and other fields that promote and prevent disease should all contribute to this research [1–4]. Premature birth, respiratory diseases, allergies, malignancies, cardiovascular disease, heightened oxidative stress, endothelial dysfunction, mental health problems, and countless other unfavorable health impacts can result from early exposure to environmental pollutants. Exposure to environmental particulate matter has been associated in numerous studies to an increased risk of cancer, organ damage, and other chronic diseases and mortality [5–10].

Glyphosate is a systemic herbicide that is not selective. Most annual and perennial plants are managed with it. Concerns about public health have grown in response to the discovery that some items contain glyphosate metabolites. Although glyphosate was found to be hazardous to organs, reproduction, and the neurological system in animal tests, there is less evidence that the

herbicide causes cancer in humans. Several environmental tests, including water, have discovered glyphosate, a herbicide widely used in agriculture. As evidence grows that glyphosate is detrimental to living creatures, questions about how to prevent its accumulation (in soils and water supplies) and how to eradicate it once it has occurred are gaining more and more attention [11–18].

Global herbicide use is projected to be between 125,000 and 130,000 metric tons per year. Glyphosate has been banned from agricultural use in over 20 nations due to concerns about its toxicity and potential health effects. However, many farmers in countries like Indonesia and Malaysia continue to use herbicides without protection, putting their health at serious risk. In light of these concerns, many experts recommend glyphosate bioremediation as an alternative to traditional physical and chemical degradation techniques. Bioremediation is an environmentally friendly and potentially safer method of dealing with glyphosate pollution in agricultural settings. It uses the metabolic activities of microbes to break down glyphosate into less dangerous compounds [19–22].

Glyphosate blocks the shikimate pathway enzyme 5-enolpyruvyl-shikimate-3-phosphate (EPSP) synthase in many organisms, including plants, bacteria, Apicomplexa, algae, and fungus. The aromatic amino acids (tryptophan, phenylalanine, and tyrosine) and vitamins (folic acid and menaquinone) rely on this system for de novo production. EPSP synthase converts two glycolytic intermediates, shikimic acid-3-phosphate (S3P) and phosphoenolpyruvate (PEP), into EPSP.

Glyphosate inhibits growth and development in these species by inhibiting EPSP synthase, which effectively affects the formation of these important chemicals [23–26]. Bacteria that can use glyphosate to bioremediate glyphosate in an Indonesian environment are great choices. In light of the extensive use of glyphosate in this paddy area, the current investigation seeks to identify any presence of these bacteria. This project aims to create viable bioremediation solutions to limit glyphosate contamination in agricultural contexts by isolating and identifying bacteria capable of digesting glyphosate.

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 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

A sterile spatula was used to collect soil samples 5 cm below the surface, which were then placed in a sterile polycarbonate container. The soil sample, weighing 1 gram, was combined with 9 mL of sterile tap water. Next, 0.2 mL of the sample was transferred and streaked onto a mineral salt agar medium (pH 7.5). This medium had the following components, 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).

With deionized water as the sole phosphate source, glyphosate was dissolved to a final concentration of 1 g/L from a stock solution of 10 g/L. At 12 g/L, glyphosate dissolved to its maximum in deionized water. Following a 15-minute autoclaving period at 121 ºC and 115 kPa, the medium was filtered with glyphosate using a 0.2-micron filter for sterilizing. Four separate colonies were observed, suggesting the existence of a bacterium capable of growth on glyphosate. Each colony was streaked into a 28 mL universal bottle containing 10 mL of the glyphosate MS medium. Then, it was incubated at room temperature for four days and shaken at 150 rpm on an orbital shaker. For the purpose of further optimization, the bacterium with the highest A600 nm measurement for glyphosate degradation was employed. This method of preserving bacterial cultures over a long period is based on [27].

The exponential growth phase was validated by an optical density (A600 nm) of about 0.6-0.8 after an overnight culture was established in LB broth at 30 °C with shaking at 150 rpm. The sterile glycerol was diluted with sterile water to make a 50% solution, which was then autoclaved at 121°C for 15 minutes. The sterile cryovials were given the appropriate labels and divided into 500 µL portions with the 50% glycerol solution. Afterwards, 500 µL of the bacterial culture was added to every cryovial, resulting in a final glycerol concentration of 25%. The mixture was quickly vortexed to ensure the cells were distributed evenly in the glycerol solution. The last step in preparing the cryovials for long-term storage was promptly moving them to a freezer set at -80°C.

Characterization of glyphosate degradation

A microplate titer method was used to conduct the studies. Using a pipette, 200 µL of the glyphosate-MSM medium—mentioned earlier—was introduced to the microplate. The microplate wells were also supplemented with 20 µL of bacterial inoculum. The microplate was covered and left undisturbed at room temperature for four days. We could assess the bacterial isolate's development during glyphosate breakdown by dissecting the effects of pH, concentration, temperature, heavy metals, inoculum size, and aeration. One factor at a time (OFAT) was the method used to determine how each factor affected growth. Finding the best isolate requires studying how several factors, including incubation time, glyphosate content, inoculum size, pH, temperature, and effects of heavy metals, play a role.

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

The bacteria was biochemically and phenotypically described using traditional morphological and biochemical techniques, following the guidelines in Bergey's Manual of Determinative Bacteriology [29]. The results were interpreted via the ABIS online system [30].

Statistical analysis

Every experiment was carried out three times to ensure the results could be reliably reproduced. The standard deviation bars show the experimental mistakes. The GraphPad Instat software was used for statistical analysis of data. We used one-way ANOVA with a 95% confidence interval to determine whether there was a statistically significant relationship.

RESULTS AND DISCUSSION

Screening of the Isolates

After the screening process, four bacterial colonies were identified that could use glyphosate as a source of phosphorus. Due to its greater absorbance value at A600 nm (**Table 1**), Isolate 1 was selected for additional partial identification as the best isolate.

Table 1. Growth of glyphosate-degrading isolates on 500 mg/L glyphosate.

Isolate	A600 nm (±standard deviation, n=3).		
1	1.47	±	0.06
2	0.38	±	0.01
3	0.76	±	0.03
4	1.12	±	0.10

Partial identification of the bacterium

The bacterium was a Gram-positive, rod-shaped bacterium. Culture, morphology, and a battery of biochemical analyses all pointed to the same bacterium, which allowed for its identification (**Table 2**) to the Bergey's Manual of Determinative Bacteriology [29] and using the ABIS online software [30]. The software gave two suggestions for the bacterial identity, *Bacillus subtilis* and *Bacillus atrophaeus*, with the same 91% homology and 92% accuracy, indicating that assignment to the species level cannot be done. Despite this, we are carrying out further molecular identification techniques by analysing the 16srRNA gene of this bacterium. The bacterium is tentatively identified as *Bacillus* sp. strain Unand1 at this juncture. Numerous bacteria from this genus are known for their ability to degrade pesticides, including glyphosate [31–34]. Hence, the assignment to the species level cannot be done at this juncture. More work, especially molecular identification techniques through comparison of the 16srRNA gene, is needed to identify this species further. Other glyphosate-degrading bacteria include *Alcaligenes* sp. [35], *Flavobacterium* sp. [36], *Bacillus megaterium* [32], *Geobacillus caldoxylosilyticus* [37], Enterobacter cloacae [38], *Rhizobium* sp. and *Agrobacterium* sp. [39] and *R. aquatilis* [40].

Effect of initial pH

The effect on glyphosate degradation was assessed over the pH scale from 4.0 to 9.0. The results show that, according to ANOVA analysis, the ideal pH range for bacterial growth is between 6.0 and 7.0 after 4 days of incubation, with growth being considerably reduced ($p<0.05$) at values higher than the optimal value (**Fig. 1).**

Table 2. Biochemical tests for *Bacillus* sp. strain Unand1.

Gram positive staining	+	Acid production from:	
Motility	+		
Growth on usual media *	+	N-Acetyl-D-Glucosamine	d
Growth at 45 ºC	+	Cellobiose	+
Growth at 65 ºC	–	Fructose	+
Hemolysis	+	L-Arabinose	+
Growth at pH 5.7	+	D-Glucose	+
Anaerobic growth	–	Glycogen	+
Growth on 7% NaCl media	+	Glycerol	+
Casein hydrolysis	+	Lactose	d
Growth in Lysozyme (0.001%)	–	meso-Inositol	+
Gelatin hydrolysis	+	D-Mannose	+
Esculin hydrolysis	+	Mannitol	+
Tyrosine degradation	–	Melezitose	–
Starch hydrolysis	+	Maltose	+
Catalase	+	Raffinose	+
Beta-galactosidase (ONPG)	+	Melibiose	d
Urease	–	Ribose	+
Oxidase	d	Rhamnose	–
Lysine decarboxylase (LDC)	–	Sorbitol	+
Arginine dehydrolase (ADH)	–	Salicin	+
Citrate utilization	+	Starch	+
Ornithine decarboxylase (ODC)	d	Sucrose	+
Nitrates reduction	+	D-Xylose	+
Egg-yolk reaction	–	Trehalose	+
Voges-Proskauer test (VP)	+		

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

Characterization of glyphosate degradation

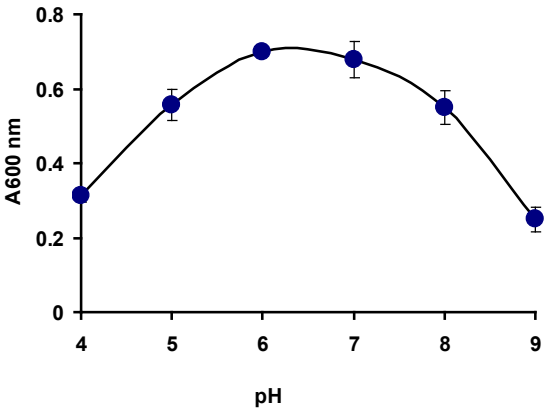

Fig 1. Growth at various initial pH on glyphosate by *Bacillus* sp. strain Unand1. Glyphosate was at 500 mg/L, at room temperature (28 °C) and shaken for four days at 150 rpm. The error bar represents the mean ± standard deviation (n=3).

Effect of glyphosate concentration on glyphosate reduction

We evaluated the effect of carbon supply on this bacteria's glyphosate degradation capacity from concentrations ranging from 0.1 to 1 g/L. The optimal glyphosate doses for growth support, according to the results, were 500 to 600 mg/L (**Fig. 2**).

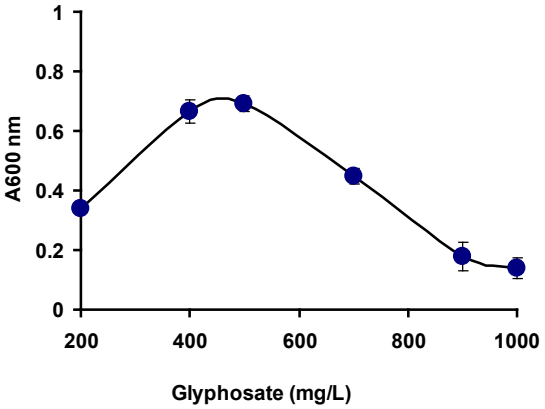

Fig 2. Growth at various concentrations of glyphosate by *Bacillus* sp. strain Unand1. pH was 7.0, growth was at room temperature (28 °C) and shaken for four days at 150 rpm.

Effect of inoculum size on Glyphosate biodegradation

To find out how different inoculum sizes affected the reduction of glyphosate, we examined them from 0.1 to 1% (v/v) of an initial stock of A600 nm of 1.0. According to the results, the growth rate was proportional to the inoculum size (**Fig. 3**).

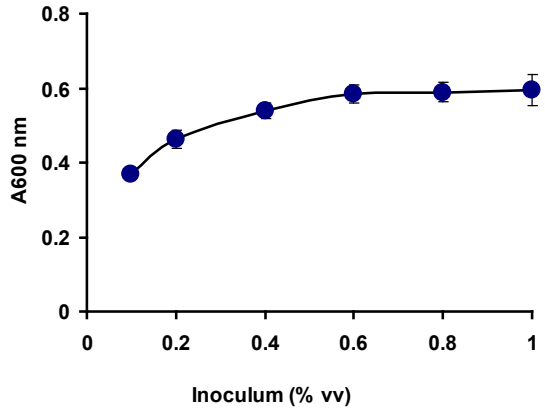

Fig 3. Growth at various inoculum size on glyphosate by *Bacillus* sp. strain Unand1. Glyphosate was at 500 mg/L, at pH 7.0, at room temperature (28 °C) and shaken for four days at 150 rpm.

Effect of temperature

The biodegradation of glyphosate was studied at temperatures ranging from 20 to 50 degrees Celsius. According to the data, the ideal temperature was 30 degrees Celsius, and a drop in growth was found at higher temperatures that was statistically significant ($p<0.05$) (**Fig. 4**).

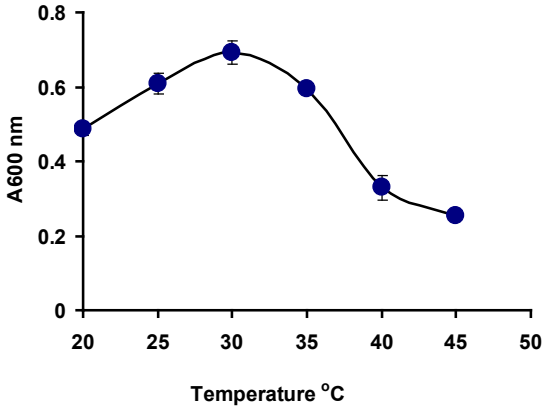

Fig 4. Growth at various temperatures on glyphosate by *Bacillus* sp. strain Unand1. Glyphosate was at 500 mg/L, pH was 7.0, inoculum size of 1% and shaken for four days at 150 rpm.

Growth of bacterium and Degradation of glyphosate

This bacterium's growth at 500 mg/L glyphosate shows a lag period that lasts about two days (**Fig. 5**). Glyphosate concentration was decreased concomitant with cellular growth. The control judged that glyphosate's abiotic Degradation was minimal.

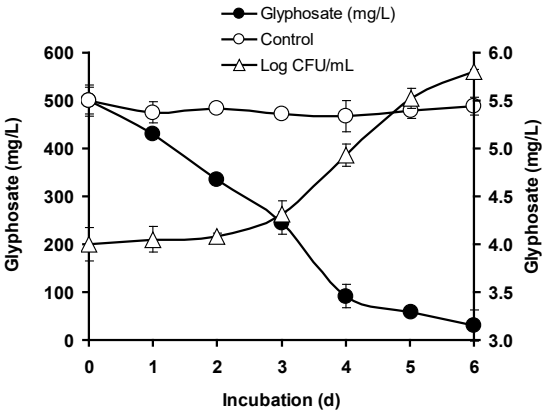

Fig. 5. Growth profile of *Bacillus* sp. strain Unand1 on 500 mg/L glyphosate and degradation profile. Each data point represents the mean ± standard deviation of three replicates.

The effect of heavy metals

As a result of heavy metals like Hg(II) and Ag(I), growth on glyphosate was inhibited by 99 and 95 percent, respectively. It is observed that other metals do not hinder growth on glyphosate (**Fig. 6**).

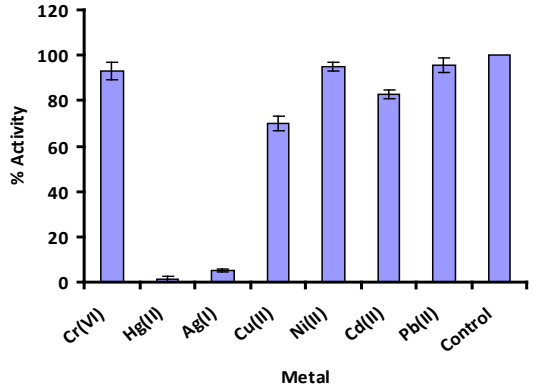

Fig. 6. Growth at various heavy metals on glyphosate by *Bacillus* sp. strain Unand1. Glyphosate was at 500 mg/L, pH was 7.0, at room temperature, inoculum size of 1% and shaken for four days at 150 rpm.

DISCUSSION

Suppose the microbial strains that are introduced are capable of entirely metabolizing glyphosate. In that case, there is a possibility that it will be possible to prevent the accumulation of potentially hazardous intermediates that can be the result of incomplete breakdown. This entire metabolic process is necessary for bioremediation activities since it guarantees that the products of the breakdown are harmless to the environment and do not include harmful substances. Maintaining a particular set of environmental parameters consistently is required during incubation. This is done to either encourage microbes' growth or keep conditions suitable for a biological reaction. This technique is essential to bioremediation because it produces an atmosphere perfect for bacteria growth.

The purpose of proper incubation is to ensure that bacteria are provided with the environmental stability required to function effectively. All living organisms, including bacteria, depend on various sustenance forms for their continued existence, growth, and metabolic processes. Chemicals such as glyphosate have the potential to influence the growth and metabolism of plants, but this is contingent on the degree to which these nutrients are easily available. Based on the research findings, it appears that the capacity of microorganisms to utilise a source of low carbon may have an inverse relationship with their growth potential. Bacteria may be able to break down pesticides such as glyphosate more effectively in environments where carbon sources are scarce. The study did not seek to evaluate the possibility that the presence of additional carbon sources could accelerate the breakdown of glyphosate. Considering that differing carbon sources may affect microorganisms' breakdown routes and effectiveness, this should be considered in future research.

Despite this, the most efficient Degradation of glyphosate occurred at dosages ranging from 500 to 700 mg/L when no other carbon sources were available. Because this research demonstrates that certain microbial strains can use glyphosate as their only carbon source, bioremediation strategies being developed to clean up glyphosate contaminations can profit from this discovery. Some bacteria can degrade higher concentrations of glyphosate. For example, it was discovered that *Acetobacter* sp. and *P. fluorescens* thrived at a concentration of 7500 parts per million (7.5 grams per liter), even though they were able to endure doses of glyphosate as high as 250,000 parts per million (250 grams per liter) [41].

Due to the fact that there is a negative association between high inoculum concentration and bacterium development, which therefore impacts glyphosate breakdown, it is extremely important to have a solid understanding of the impact that inoculum size has on glyphosate reduction. In order to ensure that the bacterial isolate can grow in the most efficient manner possible, it is essential to use the appropriate quantity of bacterial inoculum. If an insufficient amount is utilized, the nutrients in the medium will be depleted before the bacteria can ingest them, which will fail the experiment. On the other hand, a high concentration could lead to death and growth suppression due to the anticipated lack of nutrients in the environment. This would result in inadequate sustenance, which would then restrict growth [42]. The result is similar to *B. cereus* CB4, where a 5% inoculum is optimal. [33].

Due to the fact that it influences the growth and proliferation of cells in bacterial environments, the pH of a medium must be maintained at specific levels. The Degradation of glyphosate was not maintained at pH values lower than 5, most likely due to the fact that acidic circumstances are not conducive to the development of different types of bacteria. The pH of a medium is a measurement of the degree to which it is acidic, neutral, or alkaline. Like other microorganisms, bacteria require a pH that is appropriate and physiological to exist and carry out their metabolic processes. Because the pH scale is used to determine the degree to which a certain medium is acidic, neutral, or alkaline [43].

The ability of these organisms to control the pH gradient that exists between their internal and exterior habitats is essential to their ability to survive at pH levels that are higher than or lower than 5.0 [44]. *Bacillus cereus* CB4 [33] 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 [45]. 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 [34,36,39,46–52] including several glyphosate-degrading *Pseudomonas* spp. [26,32,45,53–58]

It has been discovered that a number of microorganisms can break down glyphosate at a variety of temperatures.; nevertheless, 30–35 °C seems to be the best spot. *Ochrobactrum* sp. GDOS [48] and *Pseudomonas putida* [45]. The optimal temperature for bacterial development is thirty degrees Celsius, which is a clear indicator that glyphosate is being used. The high temperature that our bacterium prefers to inhabit may have something to do with the heated environment in which it was discovered in the first place. Glyphosate degraders are primarily composed of mesophilic bacteria, and the temperature range that is suitable for their development is between 25 and 35 °C [34,36,39,46–52] and also including several glyphosate-degrading *Pseudomonas* spp. [26,32,45,53–58]. *Geobacillus caldoxylosilyticus* [37] is thermophilic glyphosate-degrading bacterium which as an optimum temperature at 60 °C.

Many bacteria of the genus *Bacillus* spp. have been found to digest glyphosate. Therefore, this study is not the first to find one (**Table 3**). The ability of these bacteria to proliferate on glyphosate is because they are resistant to the inhibition of the 5-enolpyruvylshikimate-3-phosphate synthase enzyme (EPSPS). This resistance is the product of gene mutation and duplication [25]. In line with the findings of several studies that demonstrated

a substantial reduction in the microbial population following the introduction of glyphosate into the medium culture, the very small number of strains that were isolated from the medium that contained glyphosate as the sole source of carbon or phosphorus is deemed appropriate. When considering the toxicity of synthetic medium as a consequence of the manner of action of glyphosate, which has the effect of blocking the shikimic acid pathway, this result makes perfect sense. If an organism is subjected to glyphosate, it will lose the ability to produce aromatic amino acids, ultimately resulting in the death of the organism's cells. This occurs because virtually every type of bacterium has a shikimic acid pathway.

Table 3. 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	-	[52]
Burkholderia sp. AQ5–12	6.0	30	91 and 74% of 50 ppm	Phosphate	-	[52]
Klebsiella oxytoca strain Saw-5	7.0	30	200 mg/L	Carbon	-	[59]
Pseudomonas sp.	7.0	40	High	Carbon	Cu, Zn, Pb, Hg, Ag, Fe	[54]
Bradyrhizobium sp.	6.0-7.0	30	Moderate	Phosphate Source	-	[60]
Trichosporon cutaneum	5.5-6.0	28	58%	Carbon	-	[61]
Candida tropicalis	5.5-6.0	28	76%	Carbon	-	[61]
Aspergillus niger	3.5-4.0	25	High	Carbon	Not Specified	[62]
Fusarium oxysporum	5.0-5.5	25	High	Carbon	-	[62]
Penicillium spinulosum	4.5-5.0	27	Moderate	Carbon	-	[62]
Aspergillus terreus	4.5-5.0	27	Moderate	Carbon	-	[62]
Aspergillus flavus	4.5-5.0	27	Moderate	Carbon	-	[62]
Mucor spp.	5.0-5.5	25	Moderate	Carbon	-	[62]
Rhizopus stolonifer	5.5-6.0	28	High	Carbon	-	[62]
Trichoderma koningii	5.5-6.0	28	High	Carbon	-	[62]
Trichosporon cutaneum	5.5-6.0	28	58%	Carbon	-	[61]
Enterobacter bugandensis	7.0	30	High	Carbon	Cd, Pb	[63]
Klebsiella sp.	6.5-7.5	35	High	Carbon	Cu, Zn, Pb	[64]
Arthrobacter sp.	6.5-7.0	30	High	Carbon	Cd, Pb	[65]
Bacillus sp.	7.0-7.5	37	High	Carbon	Cu, Zn	[66]
Sphingomonas sp.	6.5-7.0	28	Moderate	Carbon	Hg, Pb	[67]
Agrobacterium sp.	6.0-6.5	30	Moderate	Carbon	Cu, Zn, Pb	[62]
Burkholderia sp.	7.0-7.5	35	High	Carbon	Cd, Zn, Pb	[68]
Streptomyces sp.	6.5-7.0	28	Moderate	Carbon	Cu, Pb	[69]
Paenibacillus sp.	7.0-7.5	30	High	Carbon	Zn, Pb	[66]
Pseudomonas putida	9.0	30	1000 mg/L	Phosphate	-	[45]
Bacillus subtilis strain Bs-15	8.0	35	10,000 mg/L	Phosphate	-	[31]
Bacillus cereus CB4	6.0	35	6000 mg/L	Phosphate	-	[33]
Bacillus sp. strain Unand1	6.0 to 7.0	30	500 to 600 mg/L	Phosphate	Hg, Ag	This study

Mercury also inhibited the growth of *Sphingomonas* sp. [67] on glyphosate as a carbon source and *Pseudomonas* sp. as a phosphate source [54], which indicates that this harmful metal has a significant blocking effect on the biodegradation process. 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. The significance of this statement lies in the fact that it draws attention to a gap in the research.

Numerous studies that concentrate on disintegration frequently fail to consider the inhibiting effects of heavy metals. This omission is very concerning because numerous locations that have been contaminated with pollutants such as glyphosate are also contaminated 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. To establish strategies for environmental cleanup, it is necessary to investigate how heavy metals interact with degradation mechanisms. It is via this research that heavy metals will be prevented from impeding the breakdown of contaminants, ultimately leading to an improvement in the efficiency of efforts to clean up the environment.

CONCLUSION

In the course of the screening of bacterial isolates, it was discovered that four separate colonies were able to make use of glyphosate as a source of phosphorus. The most promising isolate was provisionally identified as *Bacillus* sp. strain Unand1 through partial identification procedures. This identification was based on the growth characteristics of the isolate. Under ideal circumstances, this Gram-positive, rod-shaped bacterium demonstrated a significant capacity for glyphosate breakdown. The research results established that *Bacillus* sp. can degrade glyphosate at a particular pH, glyphosate concentration, temperature range, and inoculum size. The bacteria showed A remarkable adaptation to these conditions, as seen by its effective destruction over the duration of the incubation period. Nevertheless, the presence of heavy metals severely reduced the

bacterium's growth and destruction capabilities. In conclusion, the findings of this work highlight the significant potential of Pseudomonas species to aid the bioremediation of ecosystems contaminated with glyphosate. According to the findings, this strain has the potential to be an effective instrument in the reduction of glyphosate pollution in agricultural contexts. This is especially true when molecular identification techniques are applied, and additional study and optimization are carried out.

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