JEBAT, 2024, Vol 7, No 2, 25-33
https://doi.org/10.54987/jebat.v7i2.1034

JOURNAL OF ENVIRONMENTAL BIOREMEDIATION AND TOXICOLOGY
Website: http://journal.hibiscuspublisher.com/index.php/JEBAT/index

Isolation and Characterization of a Glyphosate-degrading Bacterium from a Beach on the Pariaman City, West Sumatra

Rusnam[1]*, Fachri Ibrahim Nasution[1], Motharasan Manogaran[2], and Mohd Izuan Effendi Halmi[3]

[1]Department of Agricultural Engineering, Faculty of Agricultural Technology, Andalas University, Padang, 25163, Indonesia.
[2]Malaysia Genome and Vaccine Institute (MGVI) National Institute of Biotechnology Malaysia (NIBM) Jalan Bangi, 43000 Kajang, Selangor, Malaysia.
[3]Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

*Corresponding author:
Prof Dr. Rusnam
Department of Agricultural Engineering,
Faculty of Agricultural Technology,
Andalas University,
Padang, 25163,
Indonesia.
Email: rusnam_ms@yahoo.com

HISTORY

Received: 28th Aug 2024
Received in revised form: 25th Nov 2024
Accepted: 24th Dec 2024

KEYWORDS

Bioremediation
Glyphosate
Pollution
Pseudomonas sp.
Heavy metal

ABSTRACT

Bioremediation of soil pollutants, such as glyphosate, is a cost-effective and ecologically sustainable method. Glyphosate, a highly prevalent herbicide utilized for weed control, presents substantial hazards to species and their ecosystems when it becomes present in the environment. The objective of this study was to investigate the ability of a *Pseudomonas* sp. isolated from a coastal area. The isolate with the highest potential was provisionally identified as *Pseudomonas* sp. strain Andalas2016 using partial identification techniques. This bacterium shows substantial capacity for glyphosate breakdown under ideal circumstances. The experiments revealed that *Pseudomonas* sp. exhibits optimal degradation of glyphosate under the following conditions: pH 7.0, glyphosate concentration of 0.5 g/L and temperatures ranging from 30 to 35°C. The bacterium had a delay of two days when exposed to 0.5 g/L glyphosate but achieved about 90% degradation after six days of incubation. Bacterial growth was significantly reduced by heavy metals, namely Hg(II), Ag(I), and Cu(II). Growth on glyphosate was inhibited at salinity higher than seawater level. This study highlights the capacity of *Pseudomonas* sp. to remediate glyphosate-contaminated settings through bioremediation. It emphasizes the necessity for additional investigation, specifically using molecular identification techniques, to thoroughly understand and enhance this bioremediation approach. This strategy has the potential to greatly reduce the environmental effects of glyphosate pollution, while also supporting the development of healthier environments and environmentally friendly farming methods.

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 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].

On a global scale, pollution levels have reached concerning levels as a result of urbanization, industrialization, and economic growth. These factors have contributed to higher energy consumption and greater creation of trash. To comprehend the worldwide health hazards presented by many forms of environmental deterioration, such as acid deposition, water pollution, incorrect waste disposal, and greenhouse gas emissions, a thorough investigation is necessary. This research should integrate several academic disciplines and approaches, such as public health, environmental engineering, public policy, and health promotion and illness prevention. Exposure to environmental pollutants has been associated with a wide range of detrimental health consequences. Early exposure can result in a range of adverse health effects, including prematurity, respiratory disorders, allergies, malignancies, heart disease, heightened oxidative stress, endothelial dysfunction, and mental health concerns, among others. Various studies have established a correlation between exposure to environmental particulate matter and elevated risks of cancer, organ damage, chronic disorders, and mortality.

Glyphosate, a systemic herbicide, is a broad-spectrum and extensively utilized agent for controlling both annual and perennial plants. Public safety concerns have been raised due to the detection of glyphosate metabolites in agricultural products. While animal studies demonstrate that high doses of glyphosate can have harmful effects on organs, reproduction, and the neurological system, there is insufficient evidence to establish a direct link between human exposure to glyphosate and the development of cancer. However, as a result of its widespread application in farming, glyphosate has been found in several environmental samples, such as water. The growing body of research indicating the detrimental effects of glyphosate on organisms has generated interest in preventing its accumulation in soils and water sources, as well as in devising techniques to eradicate it. Bioremediation, a process that harnesses the metabolic activities of microorganisms to break down glyphosate into less toxic substances, is increasingly being recognized as a feasible and environmentally beneficial approach to combat glyphosate pollution.

The global annual herbicide use is projected to range from 125,000 to 130,000 metric tons. Glyphosate has been restricted from agricultural usage in more than 20 countries due to concerns regarding its toxicity and potential health impacts. Numerous farmers in nations such as Malaysia negligently ignore these regulations and employ glyphosate anyhow, frequently without the requisite protective equipment, thereby jeopardizing their health significantly. Due to these problems, an increasing number of specialists are suggesting the use of glyphosate bioremediation instead of traditional physical and chemical degradation methods. Bioremediation is an environmentally beneficial and potentially safer method for managing glyphosate pollution in agricultural environments. It harnesses the natural metabolic processes of microorganisms to break down glyphosate into less hazardous substances [19–22].

Glyphosate specifically targets and inhibits the enzyme 5-enolpyruvyl-shikimate-3-phosphate (EPSP) synthase in various species, including archaea, bacteria, Apicomplexa, algae, fungi, and plants. This enzyme plays a crucial role in the shikimate pathway, which is necessary for the production of aromatic amino acids such as phenylalanine, tyrosine, and tryptophan, as well as vitamins like folic acid and menaquinone. EPSP synthase catalyzes the transformation of shikimic acid-3-phosphate (S3P) and the glycolytic intermediate phosphoenolpyruvate (PEP) into EPSP. Glyphosate's suppression of EPSP synthase hampers the formation of essential chemicals, hence impeding the growth and development of these organisms [23–26]. Bacteria capable of metabolizing glyphosate are very suitable for the bioremediation of glyphosate in the environment but salinity-tolerant degraders are rarely reported. Hence, the objective of this investigation is to examine and identify potential glyphosate-degrading bacteria present in a coastal area. This project aims to create efficient bioremediation solutions to reduce glyphosate contamination in coastal areas especially areas that are rich in agricultural activities.

MATERIALS AND METHODS

Screening of Glyphosate-Degrading Isolate and Growth Medium

Soil samples were collected 5 cm of the topsoil from a beach on the Pariaman city, West Sumatra, Indonesia in 2016. One gram of the soil sample was combined with 9 mL of sterile tap water and stirred vigorously. Next, a 0.2 mL portion of this mixture was spread onto a mineral salt agar medium (pH 7.5) with the following composition in grams per liter: Glyphosate was the sole phosphorous source. The composition of the mixture includes 0.5 grams of sodium chloride (NaCl), 0.5 grams of potassium chloride (KCl), 2 grams of ammonium sulfate (NH_4SO_4), 0.2 grams of magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), 0.01 grams of calcium chloride ($CaCl_2$), 0.001 grams of iron sulfate ($FeSO_4$), and 18 grams of agar as the solidifying agent. A concentrated solution of glyphosate (10 g/L) was made in deionized water, with the final concentration of glyphosate employed as the sole source of phosphate set at 1 g/L. The medium was subsequently sterilized by autoclaving at a temperature of 121 ºC and a pressure of 115 kPa for a duration of 15 minutes. Glyphosate was introduced into the medium by means of filter sterilization using a 0.2-micron filter to avoid degradation during autoclaving.

After the period of incubation, it was seen that two separate colonies had developed, which suggests the existence of bacteria capable of breaking down glyphosate. The colonies were streaked again onto a new agar media. Afterwards, a solitary colony was moved into 10 mL of glyphosate MS medium, which was placed in 28 mL universal bottles. The bottles were then incubated at room temperature for three days on an orbital shaker set at 150 rpm. The bacteria with the highest efficiency in digesting glyphosate, as determined at A600 nm, was chosen for further improvement. To store the *Pseudomonas* bacterial cultures for an extended period, the following procedure was followed: an overnight culture was cultivated in LB broth at a temperature of 30 °C with agitation at 150 rpm until it reached the exponential growth phase, as determined by an optical density (A600 nm) of around 0.6-0.8.

A 50% glycerol solution was created by mixing sterile glycerol with sterile water and subjecting it to autoclaving at a temperature of 121°C for a duration of 15 minutes. Labelled sterile cryovials were filled with 500 µL of a 50% glycerol solution. Subsequently, 500 µL of the *Pseudomonas* culture was introduced into each cryovial, resulting in a glycerol concentration of 25%. The solution was briefly agitated in a vortex to ensure that the cells were evenly distributed throughout the glycerol solution. Subsequently, the cryovials were promptly relocated to a freezer set at a temperature of -80°C for the purpose of storing them for an extended period of time.

Characterization of Glyphosate Degradation

Glyphosate degradation was characterized through experiments employing a microplate titer technique. The glyphosate-MSM

medium, as stated earlier, was placed into the microplate using a pipette, with an addition of 200 µL of the medium each well. In addition, 20 µL of bacterial inoculum was added to each well. The microplate was subsequently sealed and left undisturbed at room temperature for a duration of five days. In order to assess the growth of the bacterial isolate while degrading glyphosate, we conducted separate examinations of many parameters, such as pH, glyphosate concentration, temperature, heavy metals, inoculum size, and aeration. The strategy, referred to as one factor at a time (OFAT), yielded a thorough comprehension of the impact of each component on the growth process. The characterisation of the optimum isolate entailed studying the effects of incubation period, glyphosate concentration, salinity (NaCl) inoculum size, heavy metal effects, pH, and temperature.

Determination of glyphosate using HPLC

Glyphosate degradation was monitored using an HPLC [27]. 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 characterized utilizing traditional morphological and biochemical approaches, as specified in Bergey's Manual of Determinative Bacteriology, to analyze its biochemical and phenotypic characteristics [28].The analysis of the findings was conducted using the ABIS web platform [29].

Statistical analysis

The studies were carried out three times to verify the results were reliable and could be reproduced. The standard deviation error bars were used to indicate experimental variation. The data were subjected to statistical analysis using the GraphPad Instat program. Statistical significance was assessed using a one-way ANOVA with a 95% confidence interval.

RESULTS AND DISCUSSION

Screening of the Isolates

Two separate colonies of bacteria capable of utilizing glyphosate as a source of phosphorus were identified throughout the screening process. The isolate with the highest absorbance value at A600 nm was selected 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 [28] and using the ABIS online software [29]. 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 Andalas2016. Numerous bacteria from this genus is known for their ability to degrade pesticides including glyphosate [30–37]. 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. [30], *Flavobacterium* sp. [38], *Bacillus megaterium* [39], *Geobacillus caldoxylosilyticus* [40], Enterobacter cloacae [36], *Rhizobium* sp. and *Agrobacterium* sp. [41] and R. aquatilis [42].

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 [43–45].

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	d
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).**

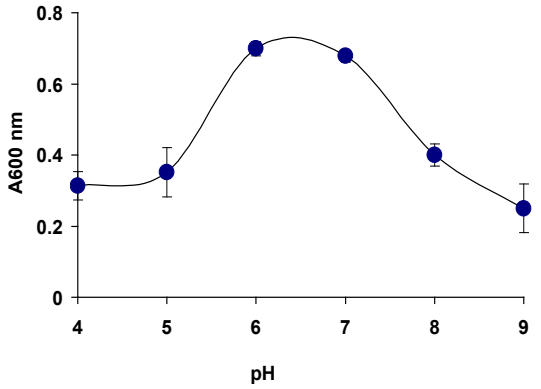

Fig 1. Effect of initial pH on glyphosate degradation by *Pseudomonas* sp. strain Andalas2016. The error bar represents the mean ± standard deviation (n=3).

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 was at 500 mg/L (**Fig. 2**).

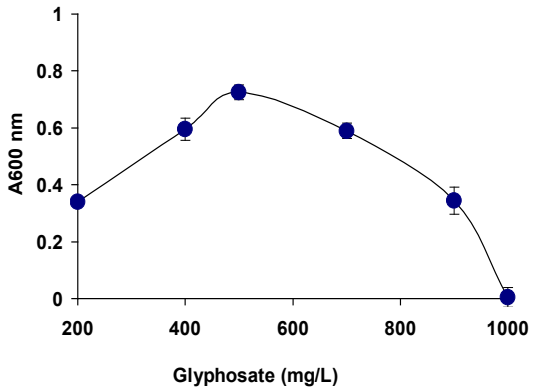

Fig 2. Effect of glyphosate concentration on glyphosate degradation by *Pseudomonas* sp. strain Andalas2016. Error bars represent mean ± standard deviation (n=3).

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 linearly, the growth was also increased linearly (**Fig. 3**).

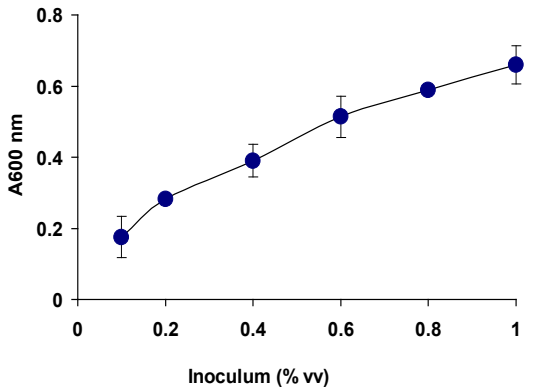

Fig 3. Effect of inoculum sizes (% v/v) on the growth of glyphosate-degrading *Pseudomonas* sp. strain Andalas2016. The error bar represents the mean ± standard deviation (n=3).

Effect of temperature

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

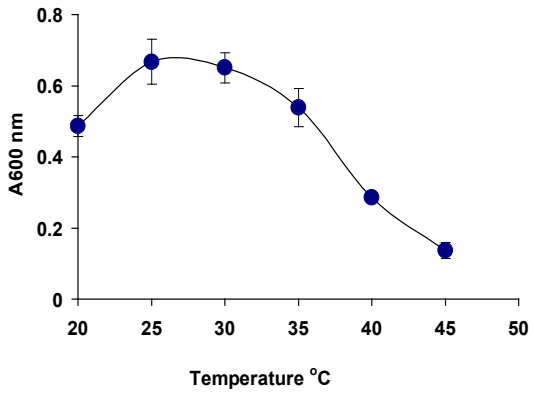

Fig 4. Effect of temperature on glyphosate degradation by *Pseudomonas* sp. strain Andalas2016. The error bar represents the mean ± standard deviation (n=3).

Growth of bacterium and degradation of glyphosate

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

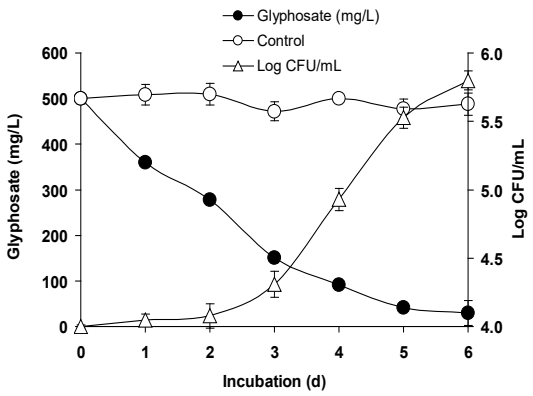

Fig. 5. Growth profile of *Pseudomonas* sp. strain Andalas2016 on 500 mg/L glyphosate. Each data point represents the mean ± standard deviation of three replicates.

The effect of heavy metals

Heavy metals such as Hg(II), Ag(I) and Cu(II) at 2 ppm caused 99, 95 and 56.7% inhibition of growth on glyphosate. Other metals are considered not inhibitory (**Fig. 6**).

The effect of salinity

The growth response of *Pseudomonas* sp. strain Andalas2016 under different salinity conditions was evaluated in the presence of 500 mg/L glyphosate. As shown in **Fig. 7**, salinity exerted a negative effect on bacterial growth. At low salinity levels, strain Andalas2016 exhibited normal growth. However, as the salinity (NaCl) increased, a progressive decline in growth was observed.

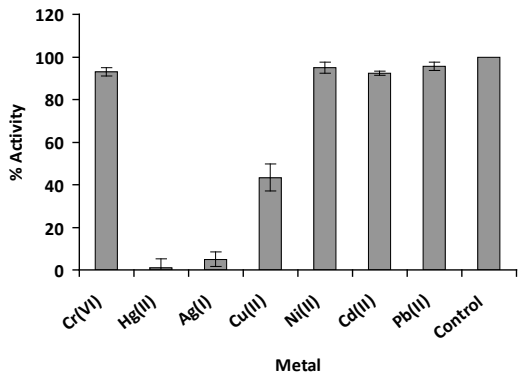

Fig. 6. Effect of 1 mg/L of heavy metals on growth of *Pseudomonas* sp. strain Andalas2016 on 500 mg/L glyphosate. Each data point represents the mean ± standard deviation of three replicates.

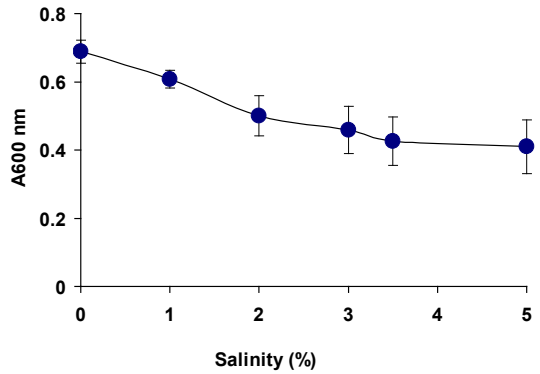

Fig. 7. Effect of salinity on the growth of *Pseudomonas* sp. strain Andalas2016 on 500 mg/L glyphosate. Each data point represents the mean ± standard deviation of three replicates.

DISCUSSION

One possible approach to prevent the build-up of dangerous byproducts caused by partial breakdown of glyphosate is to employ microbial strains that can completely metabolize the herbicide. It is essential for successful bioremediation to guarantee that these byproducts are not harmful to the environment and do not pose any toxicity. It is crucial to maintain precise environmental conditions during incubation in order to facilitate microbial growth and enhance the effectiveness of bioremediation. Optimal incubation conditions provide a favorable setting for microorganisms to flourish, guaranteeing the necessary stability for their optimal operation. Nutrients are essential for all living creatures' survival, proliferation, and metabolic functions, including microorganisms. The presence of herbicides such as glyphosate can have a major impact on the growth and metabolism of organisms, especially when it comes to the availability of essential nutrients.

Studies indicate that the capacity of microbes to exploit low-carbon sources may have an opposite effect on their development potential, suggesting that they may be more efficient at breaking down glyphosate in situations with limited carbon availability. This study did not investigate the potential for other carbon sources to speed up the breakdown of glyphosate. Future research should take into account this feature, as various carbon sources may influence the effectiveness and routes of microbial decomposition. However, the study revealed that glyphosate was most efficiently broken down at concentrations ranging from 500 to 700 mg/L in the absence of other carbon sources. This discovery emphasizes that specific microbial strains have the ability to use glyphosate as their only source of carbon, which is beneficial for bioremediation endeavors. Specific bacteria has the ability to break down elevated levels of glyphosate. For example, studies have demonstrated that both *Acetobacter* sp. and *Pseudomonas* fluorescens exhibit optimal growth at a concentration of 7500 ppm (equivalent to 7.5 g/L) and can withstand glyphosate levels as high as 250,000 ppm (equivalent to 250 g/L). This capability showcases the potential of these microbial strains to be exceptionally efficient in remediating areas with significant glyphosate contamination.

It is important to comprehend the influence of the size of the inoculum on the reduction of glyphosate. This is because there is a proven inverse relationship between a high concentration of the inoculum and the growth of bacteria, which in turn impacts the breakdown of glyphosate. Appropriate bacterial inoculum quantity is crucial for achieving optimal growth. Insufficient inoculum might deplete nutrients before they can be effectively consumed, while excessive inoculum can result in nutritional scarcity, leading to mortality and growth inhibition.

Ensuring the pH of the medium is maintained is crucial as it directly impacts the development and multiplication of bacteria. Glyphosate breakdown was hindered at pH levels below 5, presumably because acidic circumstances are not conducive to bacterial growth. Bacteria, along with other microorganisms, need on an optimal physiological pH in order to exist and perform metabolic functions. In order to withstand severe pH values, organisms must maintain a regulated pH gradient between their intracellular and exterior habitats. For instance, Bacillus cereus CB4 exhibited the most efficient breakdown of glyphosate at pH levels between 6.0 and 7.0, whereas *Pseudomonas* putida thrived better in more alkaline settings, with its best development occurring at pH 9.0. Effective breakdown of glyphosate by most glyphosate degraders, including some *Pseudomonas* spp., is dependent on a pH range that is neutral to alkaline.

Pseudomonas species possess a high degree of genetic flexibility, which boosts their ability to degrade substances through biodegradation. Recent genomic investigations have shown that gene duplication and horizontal gene transfer play a crucial role in the degradation processes of *Pseudomonas*. Their genetic adaptability enables them to thrive and operate effectively in a wide range of polluted and varied habitats. Moreover, the development of biofilms by *Pseudomonas* spp. Improves their ability to withstand adverse conditions and promotes their efficiency in breaking down substances. Biofilms serve as protective barriers, enabling microorganisms to endure adverse environments and sustain metabolic activity [46].

Understanding the importance of the amount of inoculum in reducing glyphosate is crucial since there is a clear connection between a high concentration of inoculum and the growth of bacteria, which in turn affects the breakdown of glyphosate. To ensure optimal growth of the bacterial isolate, it is necessary to use the appropriate quantity of bacterial inoculum. Inadequate inoculum quantity can result in the premature depletion of nutrients in the medium, preventing the bacteria from fully utilizing them and ultimately causing the experiment to fail. On the other hand, an excessively high concentration of inoculum can cause the death of bacteria and hinder their growth because of a lack of nutrients in the environment, leading to inadequate feeding and decreased growth. Maximizing glyphosate breakdown requires optimizing the size of the inoculum, striking a balance between adequate bacterial growth and the availability of nutrients to avoid negative effects caused by both insufficient

and excessive inoculation [47]. Ensuring the pH stability of a medium is vital since it directly impacts the development and reproduction of cells in bacterial environments. Glyphosate breakdown was not sustained at pH levels below 5, perhaps due to the unfavorable conditions for bacterial growth in acidic environments. Bacteria and other microorganisms require a certain and optimal pH level to survive and perform their metabolic functions. pH is a quantitative measure of a substance's acidity, neutrality, or alkalinity. The pH scale measures the level of acidity, neutrality, or alkalinity of a given substance [48]. Ensuring the pH stability of a medium is essential since it directly impacts the development and reproduction of cells in bacterial environments.

Glyphosate breakdown was not sustained at pH levels below 5, perhaps due to the unfavorable conditions for bacterial growth in acidic environments. Bacteria and other microorganisms require an appropriate and physiological pH level to exist and perform their metabolic functions. pH is a measurement of the acidity, neutrality, or alkalinity of a substance. The pH scale measures the level of acidity, neutrality, or alkalinity of a certain substance [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 [35]. However, there is a lack of published research on glyphosate-degrading bacteria that thrive in acidic environments. The reason for this is that most glyphosate degraders exhibit optimal breakdown rates within a pH range that is neutral to alkaline [38,41,51–58] involving various glyphosate-degrading species of *Pseudomonas* [26,32,35,37,39,59–62].

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 [26,32,35,37,39,59–62] (**Table 2**). 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].

It is crucial to ensure that microbial strains fully metabolize glyphosate in order to minimize the buildup of metabolites resulting from its breakdown. Ensuring the safety and environmental sustainability of bioremediation processes is of utmost importance. Incubation, through the creation of specific conditions, facilitates microbial development and establishes an optimal environment for efficient bioremediation. Nutrients are essential for the survival, growth, and metabolic processes of microorganisms. Chemicals like glyphosate can influence the development and metabolic processes, highlighting the need of comprehending these connections for bioremediation endeavors.

Recent research have emphasized the importance of availability in improving microbial degradation processes. Zhang et al. (2021) conducted research that showed how the inclusion of alternate carbon sources might enhance degradation efficiency by supplying metabolic energy. This highlights the need to investigate carbon sources in studies focused on improving bioremediation technologies. While the study did not specifically investigate carbon sources, it demonstrated that glyphosate was efficiently broken down at doses ranging from 500 to 700 mg/L even in the absence of such sources. This indicates that specific strains of microorganisms have the ability to use glyphosate as a source of carbon, which gives them an edge in bioremediation efforts.

The community size has an impact on the reduction of glyphosate, as there is a correlation between the initial levels of bacteria and their capacity to degrade the herbicide. Attaining equilibrium in growth is crucial. Inadequate growth leads to depletion prior to breakdown, while excessive growth results in nutritional scarcity, leading to bacterial mortality and impeding their growth. Effectively regulating the size of the inoculum is crucial for improving the success of bioremediation.

Moreover, the acidity or pH of the environment has an effect on the growth and dissemination. The degradation of glyphosate is hindered at pH values below 5 due to unfavorable circumstances for bacterial activity. Bacteria, like other microbes, necessitate specific pH values in order to survive and carry out metabolic processes. Ensuring the maintenance of pH levels is crucial for their survival in different circumstances. *Bacillus cereus* CB4 is highly proficient at decomposing substances within a pH range of 6.0–7.0, but *Pseudomonas* putida flourishes in alkaline circumstances and grows best at a pH of 9.0. The majority of bacteria that can break down glyphosate thrive in alkaline settings, particularly certain species of *Pseudomonas*.

Temperature is a factor that affects the activity of bacteria, as different types of bacteria have distinct temperature preferences. *Ochrobactrum* sp. thrives best at temperatures about 30-35°C. *Pseudomonas putida* exhibit similarities to our bacterium, as they are capable of living in similar settings. Various species of bacteria flourish within specific temperature ranges. For instance, *Pseudomonas* species are frequently observed in temperatures ranging from 25 to 35°C. *Geobacillus caldoxylosilyticus,* a bacterium that is specifically adapted to decompose glyphosate, exhibits optimal growth at approximately 60°C.

The experimental findings showed that *Pseudomonas* sp. strain Andalas2016 growth depended heavily on salinity when exposed to glyphosate because elevated salt concentrations substantially decreased bacterial population. When exposed to glyphosate, *Pseudomonas* sp. strain Andalas2016 growth depended mostly on salinity since higher salt levels significantly reduced bacterial population, according to the experimental results. The observed patterns of glyphosate degradation under different salinity levels corresponded to the findings of prior research. Yang et al. [63], for example, found that while higher salt levels (20% and 50% seawater) suppressed microbial activity and extended glyphosate half-life, mild salinization with 10% seawater greatly improved glyphosate degradation and microbial activities in glyphosate-contaminated riparian soil. Their work showed that moderate salinity maximized microbial respiration, enzymatic activity (FDA hydrolysis), and ATP levels, therefore implying that microbial activity is rather important for glyphosate degradation.

High salt concentrations create osmotic stress that restrict microbial growth and enzymatic activity which leads to reduced glyphosate degradation. The beneficial growth and activity of strain Andalas2016 at moderate salinity levels corresponds with the better biodegradation results observed in brackish conditions. The research demonstrates that optimal salinity levels are essential for achieving maximum microbial growth and glyphosate bioremediation effectiveness. This research validates that numerous *Pseudomonas* species have the ability to efficiently breakdown glyphosate, which is consistent with previous studies conducted on this subject. Their ability to withstand herbicides that target enzymes is a result of adaptations like mutations and duplications, which allow them to survive in

settings influenced by glyphosate [32]. The coexistence of different strains in situations where glyphosate is the only available supply of carbon or phosphorus is expected, as it demonstrates the influence of glyphosate on the surrounding medium by disrupting the shikimic acid pathway. This route is involved in the synthesis of amino acids.

Table 2. Characterizations of several glyphosate-degrading microrganisms.

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	-	[65]
Pseudomonas sp.	7.0	40	High	Carbon	Cu, Zn, Pb, Hg, Ag, Fe	[37]
Bradyrhizobium sp.	6.0-7.0	30	Moderate	Phosphate Source	-	[66]
Trichosporon cutaneum	5.5-6.0	28	58%	Carbon	-	[67]
Candida tropicalis	5.5-6.0	28	76%	Carbon	-	[67]
Aspergillus niger	3.5-4.0	25	High	Carbon	Not Specified	[68]
Fusarium oxysporum	5.0-5.5	25	High	Carbon	-	[68]
Penicillium spinulosum	4.5-5.0	27	Moderate	Carbon	-	[68]
Aspergillus terreus	4.5-5.0	27	Moderate	Carbon	-	[68]
Aspergillus flavus	4.5-5.0	27	Moderate	Carbon	-	[68]
Mucor spp.	5.0-5.5	25	Moderate	Carbon	-	[68]
Rhizopus stolonifer	5.5-6.0	28	High	Carbon	-	[68]
Trichoderma koningii	5.5-6.0	28	High	Carbon	-	[68]
Trichosporon cutaneum	5.5-6.0	28	58%	Carbon	-	[67]
Enterobacter bugandensis	7.0	30	High	Carbon	Cd, Pb	[69]
Klebsiella sp.	6.5-7.5	35	High	Carbon	Cu, Zn, Pb	[70]
Arthrobacter sp.	6.5-7.0	30	High	Carbon	Cd, Pb	[71]
Bacillus sp.	7.0-7.5	37	High	Carbon	Cu, Zn	[72]
Sphingomonas sp.	6.5-7.0	28	Moderate	Carbon	Hg, Pb	[73]
Agrobacterium sp.	6.0-6.5	30	Moderate	Carbon	Cu, Zn, Pb	[68]
Burkholderia sp.	7.0-7.5	35	High	Carbon	Cd, Zn, Pb	[74]
Streptomyces sp.	6.5-7.0	28	Moderate	Carbon	Cu, Pb	[75]
Paenibacillus sp.	7.0-7.5	30	High	Carbon	Zn, Pb	[72]
Pseudomonas putida	9.0	30	1000 mg/L	Phosphate	-	[35]
Bacillus subtilis strain Bs-15	8.0	35	10,000 mg/L	Phosphate	-	[76]
Bacillus sp. strain Unand1	6.0 to 7.0	30 to 35°C	500 to 600 mg/L	Phosphate	Hg and Ag	[77]
Pseudomonas sp. strain Andalas2016	7.0	25 to 30°C	500 mg/L	Phosphate	Hg, Ag and Cu	This study

Cell death in many microorganisms can occur as a consequence of this disruption. The ecological significance of *Pseudomonas* species, in relation to glyphosate, is substantial. These microorganisms aid in preservation by converting chemicals into other forms [64]. The study emphasizes the potential of this bacterium in bioremediation, indicating that additional research and molecular approaches could enhance this type of remediation, ultimately benefiting ecosystems and promoting sustainable farming practices.Growth of *Sphingomonas* sp. [73] on glyphosate as a carbon source and *Pseudomonas* sp. as a phosphate source [37] 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

While examining bacterial isolates from a paddy field, we discovered two separate colonies that have the ability to use glyphosate as a source of phosphorus. The isolate with the most potential, tentatively identified as *Pseudomonas* sp. strain Andalas2016 using incomplete identification methods, showed substantial promise in degrading glyphosate due to its growth features. Under ideal conditions, this mobile, rod-shaped, Gram-negative bacterium shown efficient breakdown of glyphosate, with specified requirements for pH, glyphosate concentration, temperature range, and inoculum size. Experimental findings shown that *Pseudomonas* sp. strain Andalas2016 exhibits strong adaptability to these circumstances, consistently maintaining effective degradation throughout the whole incubation time. Nevertheless, the bacterium's ability to grow and break down substances was greatly hindered by the existence of heavy metals, emphasizing the importance of taking into account environmental pollutants when refining bioremediation techniques. The study highlights the capacity of this bacterium to effectively clean up settings contaminated with glyphosate. The findings indicate that this particular strain has the potential to be a beneficial asset in reducing glyphosate pollution in agricultural environments. To fully maximize and exploit the bioremediation potential of this strain, it is advisable to do additional study and utilize molecular identification techniques. This will contribute to the enhancement of healthier ecosystems and the promotion of sustainable farming practices.

REFERENCES

1. Ying KK, Sulaiman MR. Toxicity effect of bisphenol-A in several animal studies: A mini review. Bioremediation Sci Technol Res. 2013 Dec 31;1(1):23–6.
2. Fardami AY, Kawo AH, Yahaya S, Lawal I, Abubakar AS, Maiyadi KA. A Review on Biosurfactant Properties, Production and Producing Microorganisms. J Biochem Microbiol Biotechnol. 2022 Jul 31;10(1):5–12.
3. Yakubu A, Uba G, Vyas A. Optimization of Culture Conditions for the Production of Alkaline Cellulase Enzyme Produced from Fusarium oxysporum VSTPDK. J Environ Microbiol Toxicol. 2021 Jul 31;9(1):3–9.
4. Mohammed UY, Hamzah AP, Abubakar S. Consistent Organochlorinated Pesticides (OPC) Residues Contamination in Beans Consumed in Gombe Metropolis Nigeria. J Environ Bioremediation Toxicol. 2021 Dec 31;4(2):1–6.
5. Vasantharaj S, Sathiyavimal S, Senthilkumar P, Kalpana VN, Rajalakshmi G, Alsehli M, et al. Enhanced photocatalytic degradation of water pollutants using bio-green synthesis of zinc oxide nanoparticles (ZnO NPs). J Environ Chem Eng. 2021;9(4).
6. Tepanosyan G, Maghakyan N, Sahakyan L, Saghatelyan A. Heavy metals pollution levels and children health risk assessment of Yerevan kindergartens soils. Ecotoxicol Environ Saf. 2017;142:257–65.
7. Khandaker MU, Asaduzzaman K, Nawi SM, Usman AR, Amin YM, Daar E, et al. Assessment of radiation and heavy metals risk due to the dietary intake of marine fishes (Rastrelliger kanagurta) from the Straits of Malacca. PLoS ONE. 2015;10(6):1–16.
8. Tavakoly Sany SB, Hashim R, Rezayi M, Salleh A, Rahman MA, Safari O, et al. Human health risk of polycyclic aromatic hydrocarbons from consumption of blood cockle and exposure to contaminated sediments and water along the Klang Strait, Malaysia. Mar Pollut Bull. 2014 Jul 15;84(1):268–79.
9. Mrema EJ, Rubino FM, Brambilla G, Moretto A, Tsatsakis AM, Colosio C. Persistent organochlorinated pesticides and mechanisms of their toxicity. Toxicology. 2013;307:74–88.
10. Grant WF. The present status of higher plant bioassays for the detection of environmental mutagens. Mutat Res Regul Pap. 1994;310(2):175–85.
11. Zheng T, Jia R, Cao L, Du J, Gu Z, He Q, et al. Effects of chronic glyphosate exposure on antioxdative status, metabolism and immune response in tilapia (GIFT, Oreochromis niloticus). Comp Biochem Physiol Part - C Toxicol Pharmacol. 2021;239.
12. Pochron S, Simon L, Mirza A, Littleton A, Sahebzada F, Yudell M. Glyphosate but not Roundup® harms earthworms (*Eisenia fetida*). Chemosphere. 2020 Feb;241:125017.
13. Nwonumara GN, Okogwu OI. Oxidative and biochemical responses as indicators of chemical stress in *Clarias gariepinus* (Burchell, 1822) juvenile exposed to glyphosate. Sci Afr. 2020;8.
14. Moraes JS, da Silva Nornberg BF, Castro MRD, Vaz BDS, Mizuschima CW, Marins LFF, et al. Zebrafish (*Danio rerio*) ability to activate ABCC transporters after exposure to glyphosate and its formulation Roundup Transorb®. Chemosphere. 2020;248.
15. Carvalho WF, Ruiz de Arcaute C, Torres L, de Melo e Silva D, Soloneski S, Larramendy ML. Genotoxicity of mixtures of glyphosate with 2,4-dichlorophenoxyacetic acid chemical forms towards *Cnesterodon decemmaculatus* (Pisces, Poeciliidae). Environ Sci Pollut Res. 2020;27(6):6515–25.
16. Weeks Santos S, Gonzalez P, Cormier B, Mazzella N, Bonnaud B, Morin S, et al. A glyphosate-based herbicide induces sub-lethal effects in early life stages and liver cell line of rainbow trout, *Oncorhynchus mykiss*. Aquat Toxicol. 2019;216.
17. Yusof S, Ismail A, Alias MS. Effect of glyphosate-based herbicide on early life stages of Java medaka (*Oryzias javanicus*): a potential tropical test fish. Mar Pollut Bull. 2014 Aug 30;85(2):494–8.
18. Kittle RP, McDermid KJ. Glyphosate herbicide toxicity to native Hawaiian macroalgal and seagrass species. J Appl Phycol. 2016 Aug 1;28(4):2597–604.
19. Rahman F, Ismail A, Yusof S, Mazlan N, Engku-Ahmad-Khairi EA. Evaluation of Glyphosate Levels in Sediments of Milky Stork Foraging Areas in Kuala Gula Bird Sanctuary, Perak, Malaysia. Pertanika J Trop Agric Sci. 2019 Aug 20;42:995–1007.
20. Mardiana-Jansar K, Ismail BS, Razak FA, Maideen HMK, Sidek HMohd, Noorani MSN, et al. Residue determination and levels of glyphosate in surface waters, sediments and soils associated with oil palm plantation in Tasik Chini, Pahang, Malaysia. AIP Conf Proc. 2014 Sep 3;1614(1):795–802.
21. Lee LJ, Ngim J. A first report of glyphosate-resistant goosegrass (Eleusine indica (L) Gaertn) in Malaysia. Pest Manag Sci. 2000;56(4):336–9.
22. Cheah UB, Kirkwood RC, Lum KY. Adsorption, desorption and mobility of four commonly used pesticides in Malaysian agricultural soils. Pestic Sci. 1997;50(1):53–63.
23. Wilson DJ, Patton S, Florova G, Hale V, Reynolds KA. The shikimic acid pathway and polyketide biosynthesis. J Ind Microbiol Biotechnol. 1998 May 1;20(5):299–303.
24. Amrhein N, Johänning D, Smart CC. A Glyphosate-Tolerant Plant Tissue Culture. In: Neumann KH, Barz W, Reinhard E, editors. Primary and Secondary Metabolism of Plant Cell Cultures. Berlin, Heidelberg: Springer; 1985. p. 356–61. (Proceedings in Life Sciences).
25. Hertel R, Gibhardt J, Martienssen M, Kuhn R, Commichau FM. Molecular mechanisms underlying glyphosate resistance in bacteria. Environ Microbiol. 2021;23(6):2891–905.
26. Schulz A, Krüper A, Amrhein N. Differential sensitivity of bacterial 5-enolpyruvylshikimate-3-phosphate synthases to the herbicide glyphosate. FEMS Microbiol Lett. 1985 Jul 1;28(3):297–301.
27. Pongraveevongsa P, Khobjai W, Wunnapuk K, Sribanditmongkol P. High-performance liquid chromatography/uv detection for determination of glyphosate in serum and gastric content. Chiang Mai Med J. 2008;47(4):155–62.
28. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergeys Man Determinative Bacteriol. 1994;
29. Costin S, Ionut S. ABIS online - bacterial identification software, http://www.tgw1916.net/bacteria_logare.html, database version: Bacillus 022012-2.10, accessed on Mar 2015. 2015.
30. Talbot HW, Johnson LM, Munnecke DM. Glyphosate utilization byPseudomonas sp. and Alcaligenes sp. isolated from environmental sources. Curr Microbiol. 1984 Sep 1;10(5):255–9.
31. Jacob GS, Garbow JR, Hallas LE, Kimack NM, Kishore GM, Schaeffer J. Metabolism of glyophosate in Pseudomonas sp. strain LBr. Appl Environ Microbiol. 1988;54(12):2953–8.
32. Penaloza-Vazquez A, Mena GL, Herrera-Estrella L, Bailey AM. Cloning and sequencing of the genes involved in glyphosate utilization by Pseudomonas pseudomallei. Appl Environ Microbiol. 1995;61(2):538–43.
33. Moneke AN, Okpala GN, Anyanwu CU. Biodegradation of glyphosate herbicide in vitro using bacterial isolates from four rice fields. Afr J Biotechnol. 2010;9(26):4067–74.
34. Lutosławski K, Ryznar-Luty A, Cibis E, Krzywonos M, Miśkiewicz T. Biodegradation of beet molasses vinasse by a mixed culture of micro organisms: Effect of aeration conditions and pH control. J Environ Sci China. 2011 Nov 1;23:1823–30.
35. Benslama O, Boulahrouf A. Isolation and characterization of glyphosate-degrading bacteria from different soils of Algeria. Afr J Miicrbiology Res. 2013 Dec 11;7:5587–95.
36. Kryuchkova YV, Burygin GL, Gogoleva NE, Gogolev YV, Chernyshova MP, Makarov OE, et al. Isolation and characterization of a glyphosate-degrading rhizosphere strain, Enterobacter cloacae K7. Microbiol Res. 2014 Jan 20;169(1):99–105.
37. Aliyu FI, Ibrahim A, Babandi A, Shehu D, Ya'u M, Babagana K, et al. Glyphosate Biodegradation by Molybdenum-Reducing Pseudomonas sp. J Environ Microbiol Toxicol. 2022 Dec 31;10(2):42–7.
38. Balthazor TM, Hallas LE. Glyphosate-degrading microorganisms from industrial activated sludge. Appl Environ Microbiol. 1986;51(2):432–4.
39. Quinn JP, Peden JMM, Dick RE. Carbon-phosphorus bond cleavage by Gram-positive and Gram-negative soil bacteria. Appl Microbiol Biotechnol. 1989;31(3):283–7.
40. Obojska A, Ternan NG, Lejczak B, Kafarski P, McMullan G. Organophosphonate utilization by the thermophile Geobacillus caldoxylosilyticus T20. Appl Environ Microbiol. 2002;68(4):2081–4.
41. Liu CM, McLean PA, Sookdeo CC, Cannon FC. Degradation of the herbicide glyphosate by members of the family Rhizobiaceae. Appl Environ Microbiol. 1991;57(6):1799–804.
42. Peng RH, Tian YS, Xiong AS, Zhao W, Fu XY, Han HJ, et al. A novel 5-enolpyruvylshikimate-3-phosphate synthase from Rahnella

aquatilis with significantly reduced glyphosate sensitivity. PloS One. 2012;7(8):e39579.
43. Sufyan AJ, Ibrahim S, Babandi A, Yakasai HM. Characterization of Butachlor Degradation by A Molybdenum-Reducing and Aniline-degrading Pseudomonas sp. J Environ Microbiol Toxicol. 2021 Dec 31;9(2):8–12.
44. Tijjani AU, Sufyan AJ, Ibrahim S, Shehu D, Ya'u M, Babagana K, et al. Characterization of Aniline Degradation by A Previously Isolated Molybdenum-reducing Pseudomonas sp. Bioremediation Sci Technol Res. 2021 Dec 31;9(2):17–20.
45. Shehu D, Muhammed YG, Abdullahi S, Ya'u M, Ibrahim S, Babandi A, et al. Isolation and Molecular Characterisation of Polycyclic Aromatic Hydrocarbons (PAHs) Degrading Bacteria from Petrochemical Contaminated Soil. Malays J Appl Sci. 2023 Oct 31;8(2):1–12.
46. Begum SS, Radha KV. Biodegradation Kinetic Studies on Phenol in Internal Draft Tube (Inverse Fluidized Bed) Biofilm Reactor Using Pseudomonas fluorescens: Performance Evaluation of Biofilm and Biomass Characteristics. Bioremediation J. 2013 Oct 2;17(4):264–77.
47. Nourouzi MM, Chuah TG, Choong TSY, Rabiei F. Modeling biodegradation and kinetics of glyphosate by artificial neural network. J Environ Sci Health B. 2012;47(5):455–65.
48. Adams MR, Little CL, Easter MC. Modelling the effect of pH, acidulant and temperature on the growth rate of *Yersinia enterocolitica*. J Appl Bacteriol. 1991;71(1):65–71.
49. Casal C, Cuaresma M, Vega JM, Vilchez C. Enhanced productivity of a lutein-enriched novel acidophile microalga grown on urea. Mar Drugs. 2011;9(1):29–42.
50. Fan J, Yang G, Zhao H, Shi G, Geng Y, Hou T, et al. Isolation, identification and characterization of a glyphosate-degrading bacterium, Bacillus cereus CB4, from soil. J Gen Appl Microbiol. 2012;58(4):263–71.
51. Sharifi Y, Pourbabaei AA, Javadi A, Abdolmohammad MH, Saffari M, Morovvati A. Biodegradation of glyphosate herbicide by Salinicoccus spp isolated from Qom Hoze-soltan lake. Iran Env Health Eng Manag J. 2015;2(1):31–6.
52. Lerbs W, Stock M, Parthier B. Physiological aspects of glyphosate degradation in Alcaligenes spec. strain GL. Arch Microbiol. 1990;153(2):146–50.
53. Hadi F, Mousavi A, Noghabi KA, Tabar HG, Salmanian AH. New bacterial strain of the genus Ochrobactrum with glyphosate-degrading activity. J Environ Sci Health - Part B Pestic Food Contam Agric Wastes. 2013;48(3):208–13.
54. Somara S, Siddavattam D. Plasmid mediated organophosphate pesticide degradation by Flavobacterium balustinum. Biochem Mol Biol Int. 1995;36(3):627–31.
55. Krzyśko-Łupicka T, Orlik A. Use of glyphosate as the sole source of phosphorus or carbon for the selection of soil-borne fungal strains capable to degrade this herbicide. Chemosphere. 1997;34(12):2601–5.
56. Manogaran M, Yasid NA, Ahmad SA. Mathematical modelling of glyphosate degradation rate by *Bacillus subtilis*. J Biochem Microbiol Biotechnol. 2017 Jul 31;5(1):21–5.
57. Manogaran M, Ahmad SA, Yasid NA, Yakasai HM, Shukor MY. Characterisation of the simultaneous molybdenum reduction and glyphosate degradation by *Burkholderia vietnamiensis* AQ5-12 and *Burkholderia* sp. AQ5-13. 3 Biotech. 2018 Feb 7;8(2):117.
58. Manogaran M, Shukor MY, Yasid NA, Johari WLW, Ahmad SA. Isolation and characterisation of glyphosate-degrading bacteria isolated from local soils in Malaysia. Rendiconti Lincei. 2017;28(3):471–9.
59. Mohy-Ud-Din W, Akhtar MJ, Bashir S, Asghar HN, Nawaz MF, Chen F. Isolation of Glyphosate-Resistant Bacterial Strains to Improve the Growth of Maize and Degrade Glyphosate under Axenic Condition. Agriculture. 2023 Apr;13(4):886.
60. Moore JK, Braymer HD, Larson AD. Isolation of a Pseudomonas sp. which utilizes the phosphonate herbicide glyphosate. Appl Environ Microbiol. 1983;46(2):316–20.
61. Dick RE, Quinn JP. Control of glyphosate uptake and metabolism in Pseudomonas sp. 4ASW. FEMS Microbiol Lett. 1995;134(2–3):177–82.
62. Dick RE, Quinn JP. Glyphosate-degrading isolates from environmental samples: occurrence and pathways of degradation. Appl Microbiol Biotechnol. 1995 Jul;43(3):545–50.
63. Yang C, Shen S, Wang M, Li J. Mild salinization stimulated glyphosate degradation and microbial activities in a riparian soil from Chongming Island, China. J Environ Biol. 2013 Apr;34(2 Spec No):367–73.
64. Chaturvedi V, Kumar A. Isolation of sodium dodecyl sulfate degrading strains from a detergent polluted pond situated in Varanasi city, India. J Cell Mol Biol. 2010;8(2):103–11.
65. Sabullah MK, Rahman MF, Ahmad SA, Sulaiman MR, Shukor MS, Shamaan NA, et al. Isolation and characterization of a molybdenum-reducing and glyphosate-degrading *Klebsiella oxytoca* strain Saw-5 in soils from Sarawak. Agrivita. 2016;38(1):1–13.
66. Hernández Guijarro K, De Gerónimo E, Erijman L. Glyphosate biodegradation potential in soil based on glycine oxidase gene (thio) from Bradyrhizobium. Curr Microbiol. 2021 May;78(5):1991–2000.
67. Stoyanova K, Gerginova M, Peneva N, Dincheva I, Alexieva Z. Biodegradation and Utilization of the Pesticides Glyphosate and Carbofuran by Two Yeast Strains. Processes. 2023 Nov 30;11(12):3343.
68. Omorinola AA, Ogunbiyi OJ, Jegede RJ, Adeleke MA, Fakolade BA. Biodegradation of glyphosate containing herbicide by soil fungi. Innovare J Life Sci. 2023 Sep 26;36–40.
69. Han H, Zhang H, Qin S, Zhang J, Yao L, Chen Z, et al. Mechanisms of Enterobacter bugandensis TJ6 immobilization of heavy metals and inhibition of Cd and Pb uptake by wheat based on metabolomics and proteomics. Chemosphere. 2021 Aug;276:130157.
70. Zhan H, Feng Y, Fan X, Chen S. Recent advances in glyphosate biodegradation. Appl Microbiol Biotechnol. 2018 Jun;102(12):5033–43.
71. Tang FHM, Jeffries TC, Vervoort RW, Conoley C, Coleman NV, Maggi F. Microcosm experiments and kinetic modeling of glyphosate biodegradation in soils and sediments. Sci Total Environ. 2019 Mar;658:105–15.
72. Wilms W, Parus A, Homa J, Batycka M, Niemczak M, Woźniak-Karczewska M, et al. Glyphosate versus glyphosate based ionic liquids: Effect of cation on glyphosate biodegradation, soxA and phnJ genes abundance and microbial populations changes during soil bioaugmentation. Chemosphere. 2023 Mar;316:137717.
73. Hernández Guijarro K, De Gerónimo E, Erijman L. Glyphosate Biodegradation Potential in Soil Based on Glycine Oxidase Gene (thiO) from Bradyrhizobium. Curr Microbiol. 2021 May;78(5):1991–2000.
74. Martins GL, de Souza AJ, Osti JF, Gontijo JB, Cherubin MR, Viana DG, et al. The role of land use, management, and microbial diversity depletion on glyphosate biodegradation in tropical soils. Environ Res. 2023 Aug 15;231:116178.
75. Zabaloy MC, Allegrini M, Hernandez Guijarro K, Behrends Kraemer F, Morrás H, Erijman L. Microbiomes and glyphosate biodegradation in edaphic and aquatic environments: recent issues and trends. World J Microbiol Biotechnol. 2022 Jun;38(6):98.
76. Yu XM, Yu T, Yin GH, Dong QL, An M, Wang HR, et al. Glyphosate biodegradation and potential soil bioremediation by Bacillus subtilis strain Bs-15. Genet Mol Res GMR. 2015 Nov 23;14(4):14717–30.
77. Rusnam, Rahman MF, Khayat ME, Nasution FI, Umar AM, Yakasai HM. Characterisation of a Bacillus sp. Isolated from Soils Near Lake Maninjau Capable of Degrading Glyphosate. J Environ Microbiol Toxicol. 2023 Jul 31;11(1):69–76.