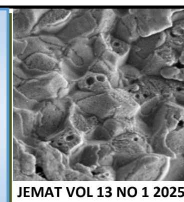
JEMAT, 2025, Vol 13, No 1, 33-38
https://doi.org/10.54987/jemat.v13i1.1109

JOURNAL OF ENVIRONMENTAL MICROBIOLOGY AND TOXICOLOGY

Website: http://journal.hibiscuspublisher.com/index.php/JEMAT/index



Isolation and Characterization of Indigenous Gram-Positive Bacteria with Glyphosate-Degrading Potential from Agricultural Soil

Annmarie Alexander[1], Jualang Azlan Gansau[1,2], Mohd Yunus Shukor[3], Nur Adeela Yasid[3] and Hartinie Marbawi[1,2]*

[1]Biotechnology Program, Faculty of Science and Technology, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia.
[2]BioAgriTech Research Group, Faculty of Science and Technology, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia.
[3]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

*Corresponding author:
Hartinie Marbawi,
Biotechnology Program,
Faculty of Science and Technology,
Universiti Malaysia Sabah,
88400 Kota Kinabalu,
Sabah,
Malaysia.
Email: hartinie@ums.edu.my

History

Received: 19th March 2025
Received in revised form: 21st May 2025
Accepted: 10th June 2025

Keywords

Bioremediation
Biodegradation
Glyphosate
Isolation
Pesticide

Abstract

Glyphosate remains one of the most extensively applied herbicides worldwide; however, its persistence in the environment raises concerns regarding ecological safety and human health, underscoring the need for sustainable remediation approaches. This study focused on isolating and characterising indigenous soil bacteria from Kota Marudu, Sabah, agricultural soil with the capacity to degrade glyphosate-based herbicides. Soil samples were enriched in minimal salt medium (MSM) supplemented with Roundup for seven days, after which bacterial isolates were screened for tolerance across different Roundup concentrations. Morphological and biochemical profiling was conducted, and partial Identification was achieved using the ABIS online platform. Biodegradation efficiency was evaluated through degradation assays. Three isolates, designated KM1, KM2, and KM4, exhibited tolerance to Roundup concentrations up to 6 mL/L (v/v). Gram staining confirmed all isolates as Gram-positive; KM1 displayed a rod-shaped morphology, whereas KM2 and KM4 were coccoid. Biochemically, KM1 tested positive for catalase activity, all sugar fermentations, the Voges–Proskauer test, and citrate utilisation. KM2 and KM4 were catalase-positive, Voges-Proskauer-positive, and citrate-positive but failed to ferment sucrose while fermenting other tested sugars. ABIS-based Identification suggested KM1 belonged to the genus *Bacillus*, while KM2 and KM4 were classified as *Staphylococcus* sp. After three days, glyphosate degradation efficiencies reached 82.8%, 83.1%, and 80.1% for KM1, KM2, and KM4, respectively. These findings highlight the potential of indigenous Gram-positive bacteria in glyphosate biodegradation. Further molecular Identification, optimisation of degradation parameters, and evaluation under field-relevant conditions are warranted to assess their applicability in agricultural bioremediation strategies.

INTRODUCTION

Glyphosate is the world's most widely used herbicide because it is both affordable and effective across many weed types. It plays a key role in modern farming, especially with genetically engineered glyphosate-resistant crops. However, its widespread use has raised concerns about environmental persistence and potential risks to non-target organisms, ecosystems, and human health (Gandhi et al., 2021). Glyphosate degradation in soil proceeds at a slow pace, ranging from 7 to 60 days, depending on environmental conditions [1]. Toxic metabolites such as AMPA can persist in the environment for years, raising environmental concerns. As a result, there is growing interest in developing sustainable and eco-friendly methods to remediate glyphosate contamination. One of the promising strategies is microbial biodegradation because most soil bacteria have developed mechanisms for the metabolism of glyphosate as a source of phosphorus[2]. Soil microbial communities can vary significantly based on various environmental factors, which may result in the discovery of novel glyphosate-degrading isolates with unique metabolic capabilities [3]. Several glyphosate-degrading bacteria, including species from the *Bacillus*, *Pseudomonas*, and *Burkholderia* genera, have been identified, suggesting bioremediation is a feasible approach to reducing

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

glyphosate's environmental effects [4]. To address these concerns and consider Sabah as a hotspot for microbiota biodiversity, this work attempted to isolate and characterise glyphosate-degrading bacteria found in agricultural soils in Kota Marudu, Sabah. Identifying efficient bacterial strains in this study may help develop sustainable bioremediation methods to reduce the herbicide's environmental impact.

MATERIALS AND METHODS

Chemicals and stock solution

The isopropylamine salt of glyphosate, marketed as Roundup® (containing 450 g/L active ingredient; Monsanto), was obtained from a local agricultural supply retailer in Kota Kinabalu. Technical-grade glyphosate (N-(phosphonomethyl)glycine, 95% purity) was sourced from Zhengzhou Delong Chemical Co., Ltd. All chemicals utilised in this study were of analytical grade. Glyphosate stock solutions were made by dissolving technical-grade glyphosate in deionised water, with fresh solutions prepared for each test. Solution pH was adjusted using 0.1 M NaOH or HCl as required.

Experimental soil

Soil samples were collected at five agricultural areas in Kota Marudu. These areas had a wide variety of crops, including corn, oil palm, and paddy fields. The sampling sites were chosen based on a glyphosate treatment history of at least three years to enrich for microbes with potential degradation capabilities and ensure a broad representation of bacterial diversity [5]. Samples were taken from 5–10 cm of topsoil, a layer prone to glyphosate contamination and high microbial activity [6]. Samples were stored in sterile plastic bags and stored at 4°C until use.

Enrichment and isolation of glyphosate-degrading strains

Mineral salt medium 2 (MSM2) pH (7.0 to 7.2) containing glucose (10), NaCl (0.5), KCl (0.5), NH_4SO_4 (2), $MgSO_4 \cdot 7H_2O$ (0.2), $CaCl_2$ (0.01), and $FeSO_4 \cdot 7H_2O$ (0.001) in grams per litre of distilled water was used to enrich and isolate bacteria. Glyphosate was employed as the sole phosphorus source. Enrichment and isolation were performed using a method adapted from [7]. Around 5.0 g of each soil sample was added to 95 mL of MSM2 medium in 250 mL flasks with the addition of filter-sterilised 1 mL/L Roundup (0.2 µm filter). The flasks were incubated in the dark at 28°C with agitation at 150 rpm for seven days. To isolate individual colonies, a 0.2 mL aliquot of enriched culture was transferred and streaked onto mineral salt medium agar plates containing 1 mL/L Roundup (pH 7.0). The agar plates were kept at 28°C for three days. Six distinct colonies (KM1-KM6) were found to contain glyphosate-degrading bacteria. These colonies were subcultured on fresh agar media to produce pure cultures. A single colony was placed into 25 mL of MSM media in a 50 mL Erlenmeyer flask and cultured at room temperature for 3 days on an orbital shaker at 150 rpm before screening.

Screening of bacterial isolates for glyphosate-tolerance

The tolerance of bacterial isolates to a glyphosate-based herbicide (Roundup) was evaluated in a three-stage screening process: primary screening (1 mL/L Roundup), secondary screening (3 mL/L Roundup), and tertiary screening (6 mL/L Roundup). Minimal salt medium 2 (MSM2) was used for all experiments. Bacterial growth was measured by the spread plate method following a 10-fold serial dilution. Each dilution (100 µL) was plated on MSM2 agar and incubated at 28°C for 24 hours. Counts of colony-forming units per millilitre (CFU/mL) were performed every day for seven days. At each stage, isolates displaying low growth were eliminated, while high-tolerance strains proceeded to further screening and characterisation.

Morphological characterisation

Colony morphology was examined on MSM2 agar plates for size, shape, pigmentation, and margin characteristics. Cell morphology and Gram staining reaction were determined by light microscopy (100X magnification). Cell shapes were recorded, and Gram-positive or Gram-negative status was indicated.

Biochemical characterisation

Standard tests were used for biochemical profiles, such as catalase, oxidase, Voges-Proskauer (VP), citrate utilisation, and sugar fermentation assays (fructose, glucose, glycerol, and sucrose). Catalase activity was measured by the generation of oxygen bubbles when exposed to 3% hydrogen peroxide. Oxidase activity was determined using the oxidase reagent. Standard microbiological precautions were followed when performing VP and citrate utilisation tests. Sugar fermentation patterns were determined in the phenol red carbohydrate broth [7].

Preliminary Identification using ABIS Online

The biochemical profiles of the isolates were analysed using the ABIS online identification platform (Advanced Bacterial Identification Software; https://www.tgw1916.net/ABIS/bacteria_abis.html). Results were expressed as similarity percentage, probability percentage, and matrix integrity. Species-level identifications were considered tentative and subject to further confirmation.

Biodegradation Analysis

Biodegradation assays in accordance with[8] were performed in flasks containing 50 mL of MSM2 (pH 7.0) supplemented with 50 mg/L glyphosate and inoculated with a 2% (v/v) bacterial inoculum. Cultures were incubated at room temperature and agitated at 150 rpm for three days. Glyphosate was quantified by collecting 1.5 mL samples every 24 hours, which were subsequently centrifuged at 5000 rpm for 30 minutes to remove cells. The obtained cell-free supernatant was used for glyphosate determination.

Glyphosate determination

Residual glyphosate concentrations were determined using a colorimetric method as previously described by [9] with minor modifications. Absorbance was measured at 570 nm employing a microplate reader (Thermo Scientific, Multiskan Go, Finland). The percentage of glyphosate removal was calculated using the following equation:

$$\text{Glyphosate Removal (\%)} = [(C_I - C_F)/C_F] \times 100$$

Where,
C_I is the initial concentration of glyphosate, which is the initial concentration of glyphosate.
C_F is the concentration of glyphosate at a later time point.

RESULTS

Isolation and Screening of Glyphosate Tolerance

Six bacterial isolates (KM1–KM6) were successfully isolated from Kota Marudu agricultural soils. The growth performance of the bacterial isolates was evaluated under three concentrations of glyphosate-based herbicide (Roundup)—1 mL/L, 3 mL/L, and 6 mL/L—over a seven-day incubation period (**Figs. 1a–c**). In primary screening (1 mL/L), KM1, KM2, and KM4 reached 1.2–1.6 × 10^8 CFU/mL by day 7, while KM3, KM5, and KM6 showed

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

lower tolerance with growth under 0.5 × 10^8 CFU/mL. A high CFU/mL result shows a large number of viable cells per millilitre that can reproduce in the agar medium [11]. This suggests that the bacteria were able to use glyphosate excessively for their growth. During secondary screening (3 mL/L), KM1 maintained the greatest growth rate (~1.2 × 10^8 CFU/mL), followed by KM2 and KM4 (~1.0 × 10^8 CFU/mL). KM5 grew slowly but steadily, while other isolates showed minimal growth. According to [12], bacteria can develop glyphosate resistance by modifying the EPSPS gene. This modification allowed the bacteria to grow better in the presence of a greater glyphosate concentration, in this case at 3 mL/L.

At the tertiary screening (6 mL/L), KM1, KM2, KM4, and KM5 survived. Growth increased during the first two days and then reached a steady state. KM1 and KM2 maintained the highest cell densities (~3.0 × 10^8 CFU/mL), while KM4 and KM5 showed moderate levels (~2.0 × 10^8 CFU/mL). Glyphosate has a bacteriostatic effect on microorganisms, inhibiting bacterial growth by depriving them of aromatic amino acids ([13] and [14]), This stops bacterial growth, which makes the bacteria enter a state of dormancy. In order to live at high levels of glyphosate, which may be harmful to the isolates, the bacterium defends itself while in dormancy. Glyphosate thus causes stress to bacteria, which makes them metabolically inactive and dormant. These findings suggest glyphosate tolerance in KM1, KM2, and KM4, which could be attributed to physiological adaptations for survival under high-oxidative-stress conditions. Therefore, the three isolates were chosen for further Identification and biodegradation tests.

Morphological and Biochemical Characterisation of Isolates
Based on morphological characteristics, KM1 was identified as a Gram-positive rod, whereas KM2 and KM4 were classified as Gram-positive cocci (**Fig. 2**). Biochemical tests showed that KM1, KM2, and KM4 shared positive catalase and Voges-Proskauer reactions, along with glucose, fructose, and glycerol fermentation ability (**Table 1**).

Table 1. Result for biochemical test of the isolates.

Biochemical Test	Isolates		
	KM1	KM2	KM4
Oxidase Test	-	-	-
Catalase Test	+	+	+
Citrate Test	+	+	+
Indole Test	-	-	-
Voges-Proskauer Test	+	+	+
Sugar Fermentation Test			
Glucose	+	+	+
Sucrose	+	-	-
Fructose	+	+	+
Glycerol	+	+	+

+ = Positive Test, - = Negative Test

All three isolates (KM1, KM2, KM4) exhibited negative oxidase activity, consistent with previous reports of glyphosate-degrading bacteria such as *Enterobacter aerogenes*, *Stenotrophomonas acidaminiphila* Y4B, *Burkholderia* sp., and *Comamonas odontotermitis* P2 [15–18]. All isolates were catalase-positive, indicating the presence of catalase enzymes capable of decomposing hydrogen peroxide into water and oxygen. This trait is often linked to oxidative stress tolerance and has been documented in other glyphosate-degrading bacteria, including *Burkholderia* sp., *Salinicoccus* spp., and *Pseudomonas aeruginosa* ([17], [19]).

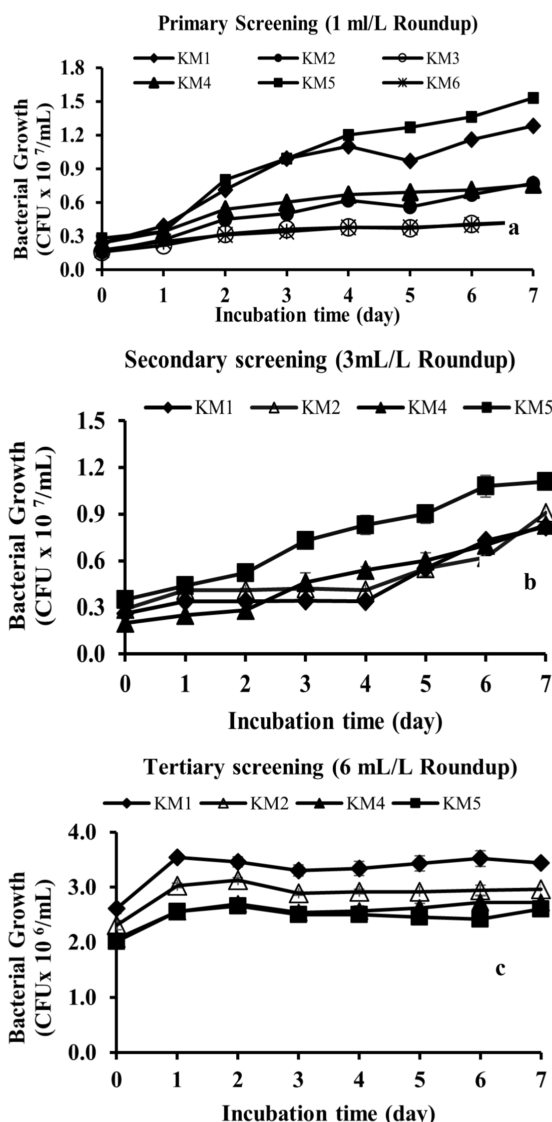


Fig. 1. Screening of bacterial tolerance to glyphosate-based herbicide, Roundup in MSM2 medium, pH 7, 150 RPM agitation (a) primary screening in 1 mL/L (b) secondary screening in 3 ml/L (c) tertiary screening in 6 ml/L.

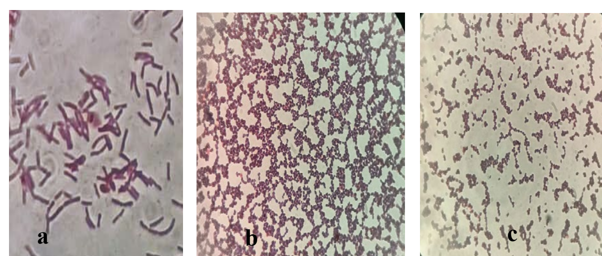


Fig. 2. Result of bacterial gram staining and morphological observation (100X magnification). (A) KM1-rod shaped (B) KM2, cocci-shaped (C) KM4 cocci-shaped.

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

Positive citrate utilisation in all isolates confirms their ability to use citrate and inorganic ammonium salts as sole carbon and nitrogen sources, resulting in an alkaline shift in the medium, as also observed in *Burkholderia vietnamiensis*, *Azotobacter* sp., and *Pseudomonas aeruginosa* [20,21]. All isolates were indole-negative, indicating the absence of tryptophanase and thus an inability to degrade tryptophan into indole. Similar results have been reported for *Stenotrophomonas acidaminiphila* Y4B, *Bacillus aryabhattai*, and *Burkholderia cepacia* PSBB1 [16,22,23].

The Voges–Proskauer test yielded weak but positive reactions in all isolates, suggesting limited production of acetoin from glucose fermentation, consistent with observations in *Enterobacter* sp. Bisph2 and *Klebsiella oxytoca* SAW-5 ([24,25]. Sugar fermentation patterns revealed that all isolates fermented glucose, fructose, and glycerol. Only KM1 fermented sucrose, while KM2 and KM4 did not. These patterns align with known variations in carbohydrate utilisation among glyphosate degraders, with positive fermentation of these sugars previously reported in *Burkholderia vietnamiensis*, *Bacillus aryabhattai*, *Klebsiella oxytoca*, and *Azotobacter* sp. [20–22]. Such distinct fermentation profiles are useful for strain differentiation and may reflect metabolic versatility relevant to glyphosate degradation.

Preliminary Identification using ABIS online

Biochemical Identification of the bacterial isolates using ABIS online software confirmed their classification into two genera: *Staphylococcus* (isolates KM2 and KM4) and *Bacillus* (isolate KM1). The complete identification data, including similarity, probability, and matrix integrity, are summarised in **Table 2**.

Table 2. Biochemical Identification of bacterial isolates using ABIS online software.

Isolate Code	Genus & Probable Species	Similarity (%)	Probability (%)	Matrix Integrity (%)
KM1	*Bacillus subtilis* (possibility of *B. atrophaeus* / *B. vallismortis*)	94.5	17.3	100
KM1	*Bacillus licheniformis*	94.5	17.3	100
KM1	*Bacillus cereus*	94.5	17.3	100
KM2 / KM4	*Staphylococcus schleiferi* subsp. *schleiferi*	99.0	39.9	100
KM2 / KM4	*Staphylococcus cohnii* subsp. *cohnii*	88.1	40.7	100

The KM1 isolate was assigned to the *Bacillus subtilis* group, which included three species with identical similarity (94.5%) and probability (17.3%) values: *B. subtilis* (with the possibility of *B. atrophaeus* or *B. vallismortis*), *B. licheniformis, and B. cereus*. The KM2 and KM4 isolates exhibited the greatest resemblance to *Staphylococcus schleiferi* subsp. *schleiferi* (99.0%, probability: 39.9%, matrix integrity: 100%). Other closely related matches included *S. cohnii* subsp. *cohnii* (88.1%, probability: 40.7%). ABIS online software identifies bacteria by comparing their biochemical and morphological data to a standard taxonomy database. The matrix integrity that is near 100% indicates the high accuracy of the bacterial Identification. It has been used in several research projects, including the Identification of dye-degrading bacteria [26] and an acrylamide-degrading bacterium from volcanic soil [27]. The reliability of ABIS identifications has been supported by cross-verification with Apiweb™, which reports a high rate of concordance [28]. These findings suggest that the ABIS algorithm is effective for preliminary taxonomic classification, offering a rapid and accessible approach for bacterial Identification in environmental microbiology studies. The biochemical profiles of both isolates are consistent with their respective genera, but phenotypic methods alone cannot conclusively determine their taxonomic classification. The *Bacillus* isolate's overlapping features within the *B. subtilis* complex demand molecular methods such as 16S rRNA gene sequencing, multilocus sequence typing (MLST), and whole-genome analysis. Similarly, the *Staphylococcus* isolates' high similarity score to *S. schleiferi* subsp. *schleiferi* supports tentative Identification, but the moderate probability values emphasise the significance of molecular confirmation. Applying the molecular identification approach will improve classification accuracy and confidence, thereby facilitating more reliable downstream interpretation of biodegradation potential.

While members of the *B. subtilis* complex are well-regarded for their metabolic versatility and environmental resilience [29,22,30], the role of *Staphylococcus* spp. in glyphosate degradation remains largely underexplored. Together, these findings not only broaden the spectrum of bacterial taxa known to participate in herbicide biodegradation but also provide a valuable foundation for developing microbial strategies aimed at mitigating glyphosate pollution in agricultural environments. Their tolerance to glyphosate strengthens the case for further investigation into their enzymatic degradation pathways, which could lead to the development of microbial consortia for field-scale bioremediation in herbicide-impacted agricultural soils.

Glyphosate Biodegradation Efficiency

The glyphosate degradation potential of *Bacillus* sp. KM1, *Staphylococcus* sp. KM2, and *Staphylococcus* sp. KM4 was tested throughout a 72-hour incubation period (**Fig. 3**). All three isolates demonstrated increasing herbicide degradation, reaching peak efficiencies between 48 and 72 h. At 24 hours, degradation rates ranged from 38.5% (*Bacillus* sp. KM1) to 46.2% (*Staphylococcus* sp. KM2), indicating rapid initial adaptation to glyphosate as a substrate. By 48 hours, all isolates had degraded more than 80%, with KM1 and KM2 reaching 83.1% and KM4 reaching 82.8%. Slight decreases were observed at 72 hours, most likely due to nutrient depletion or accumulation of intermediate metabolites that can inhibit further degradation. The high degradation efficiencies observed here, particularly within the first 48 h, highlight the potential of these indigenous isolates for application in glyphosate bioremediation strategies in agricultural soils. The findings are consistent with previous reports indicating that native bacteria adapted to herbicide-contaminated environments often display accelerated degradation kinetics due to prior exposure and selective enrichment [31,32].

Manogaran et al. [17] reported that *Burkholderia vietnamiensis* could degrade at least 80% of 50 ppm glyphosate under optimised conditions. In comparison, isolates KM1, KM2, and KM4 achieved an average degradation efficiency of 82% under non-optimised conditions, suggesting strong intrinsic glyphosate-degrading capabilities. Previous studies have identified other highly efficient glyphosate degraders, including *Bacillus cereus* CB4, which degraded 94.47% of glyphosate [30], and *Comamonas odontotermitis* P2, which achieved 90% degradation [18]. Reported degradation efficiencies among glyphosate-degrading bacteria vary widely, ranging from 54% to 100% [33], [34], reflecting the influence of factors such as glyphosate concentration, temperature, and bacterial strain-specific traits [35]. The comparable degradation pattern among the isolates suggests that both species may have enzyme systems capable of efficiently cleaving the C-P bond in glyphosate, either via the C-P lyase pathway or the glyphosate oxidoreductase route [36]. *Bacillus* species, notably *B. subtilis*, are well known for their high efficiency in glyphosate degradation, with some strains capable of tolerating and thriving at concentrations of up to

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

https://doi.org/10.54987/jemat.v13i1.1109

40,000 mg/L [5]. The ability of *Staphylococcus* spp. to degrade glyphosate is significant, as this genus is less frequently reported in glyphosate bioremediation studies compared to *Bacillus* spp. Their ability to adapt to high herbicide stress, combined with rapid resistance evolution [37] and multifunctional roles in bioremediation and plant growth promotion [38,39], highlights their importance as promising candidates for sustainable glyphosate remediation strategies.

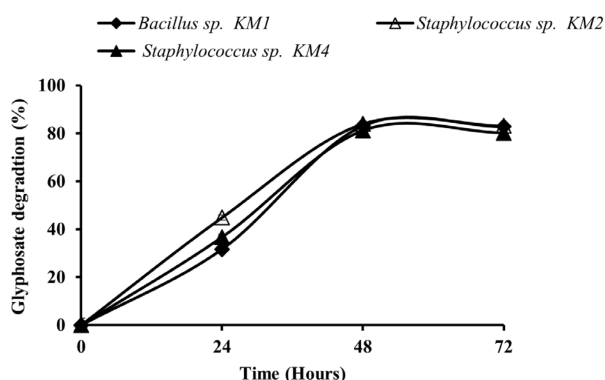


Fig. 3. Biodegradation efficiency of the three strains in MSM media supplemented with 50 mg/l glyphosate within 72 hours of incubation.

CONCLUSION

The present study identified three indigenous bacterial isolates, KM1 (Bacillus sp) and KM2/KM4 (Staphylococcus sp.), that demonstrated significant tolerance to glyphosate and substantial degradation efficiencies under non-optimised conditions. Morphological, biochemical, and ABIS-based profiling provided preliminary taxonomic placement and revealed oxidative stress adaptation traits that likely contribute to their persistence in glyphosate-rich environments. Comparative analysis with previously reported degraders shows that these isolates possess competitive biodegradation capacities, which have the potential to be improved further under optimal environmental conditions. However, definitive species-level classification requires advanced molecular identification methods such as 16S rRNA sequencing, multilocus sequence typing, or whole-genome analysis to ensure accurate taxonomy and a deeper understanding of their metabolic pathways. This confirmation will be critical for verifying their function in glyphosate biodegradation and realising their full potential in the development of tailored, sustainable bioremediation solutions for glyphosate-contaminated agricultural soils.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

The authors express their sincere gratitude to the Faculty of Science and Technology, Universiti Malaysia Sabah, and the Bioremediation Biomonitoring and Ecotoxicology Lab, Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, for providing research facilities and laboratory support throughout this study. Special thanks are extended to the technical staff of the Faculty of Science and Technology for their assistance in microbial culture handling and biochemical analyses.

REFERENCES

1. Tzanetou E, Karasali H. Glyphosate Residues in Soil and Air: An Integrated Review. IntechOpen. 2020;33.
2. Duke SO. Glyphosate: environmental fate and impact. Weed Sci. 2020 May;68(3):201–7.
3. Guijarro KH, Aparicio V, De Gerónimo E, Castellote M, Figuerola EL, Costa JL, et al. Soil microbial communities and glyphosate decay in soils with different herbicide application history. Sci Total Environ. 2018 Sept 1;634:974–82.
4. Chen Y, Chen WJ, Huang Y, Li J, Zhong J, Zhang W, et al. Insights into the microbial degradation and resistance mechanisms of glyphosate. Environ Res. 2022 Dec 1;215:114153.
5. 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. 2015;14(4):14717–30.
6. Zhao M, Wang M, Zhao Y, Hu N, Qin L, Ren Z, et al. Soil microbial abundance was more affected by soil depth than the altitude in peatlands. Front Microbiol. 2022 Nov 9;13:1068540.
7. Cappuccino JG, Welsh CT. Microbiology: A Laboratory Manual . Pearson Education, 2016. Available from: https://books.google.com.my/books?id=OtF5CwAAQBAJ
8. Tazdaït D, Salah R, Grib H, Abdi N, Mameri N. Kinetic study on biodegradation of glyphosate with unacclimated activated sludge. Int J Environ Health Res. 2018 July 4;28(4):448–59.
9. Bhaskara BL, Nagaraja P. Direct Sensitive Spectrophotometric Determination of Glyphosate by Using Ninhydrin as a Chromogenic Reagent in Formulations and Environmental Water Samples. Helv Chim Acta. 2006 Nov;89(11):2686–93.
10. Hartline R. Determination of Bacterial Numbers . Biology LibreTexts. 2022 [cited 2024 Jan 8]. Available from: https://bio.libretexts.org/Bookshelves/Microbiology/Microbiology_Laboratory_Manual_(Hartline)/01%3A_Labs/1.15%3A_Determination_of_Bacterial_Numbers
11. Sankaranarayanan K, Ramachandran RP, Sundararajan R. Electrically-enhanced proliferation control of cancer-stem-cells-like adult human mesenchymal stem cells – a novel modality of treatment. In: Electroporation-Based Therapies for Cancer . Elsevier; 2014 [cited 2024 Jan 11]. p. 127–59. Available from: https://linkinghub.elsevier.com/retrieve/pii/B978190756815250006 6
12. Rainio MJ, Ruuskanen S, Helander M, Saikkonen K, Saloniemi I, Puigbò P. Adaptation of bacteria to glyphosate: a microevolutionary perspective of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase. Environ Microbiol Rep. 2021 June;13(3):309–16.
13. Ospino K, Spira B. Glyphosate affects persistence and tolerance but not antibiotic resistance. BMC Microbiol. 2023 Mar 7;23:61.
14. Pöppe J, Bote K, Merle R, Makarova O, Roesler U. Minimum inhibitory concentration of glyphosate and a glyphosate-containing herbicide in *Salmonella* enterica isolates originating from different time periods, hosts, and serovars. Eur J Microbiol Immunol. 2019 June;9(2):35–41.
15. Sabullah MK, Rahman MF, Ahmad SA, Sulaiman MR, Shukor MS, Shamaan NA, et al. Assessing Resistance and Bioremediation Ability of *Enterobacter* sp. Strain Saw-1 on Molybdenum in Various Heavy Metals and Pesticides. J Math Fundam Sci. 2017 Oct 3;49(2):193–210.
16. Li J, Chen WJ, Zhang W, Zhang Y, Lei Q, Wu S, et al. Effects of Free or Immobilized Bacterium *Stenotrophomonas acidaminiphila* Y4B on Glyphosate Degradation Performance and Indigenous Microbial Community Structure. J Agric Food Chem. 2022 Nov 2;70(43):13945–58.
17. Manogaran M, Shukor MY, Yasid NA, Khalil KA, Ahmad SA. Optimisation of culture composition for glyphosate degradation by Burkholderia vietnamiensis strain AQ5-12. 3 Biotech. 2018 Feb;8(2):108.
18. Firdous S, Iqbal S, Anwar S. Optimization and modeling of glyphosate biodegradation by a novel *Comamonas odontotermitis* P2 through response surface methodology. Pedosphere. 2020 Oct 1;30(5):618–27.
19. Sharifi Y, Pourbabaei AA, Javadi A, Abdolmohammadi MH, Saffari M, Morovvati A. Biodegradation of glyphosate herbicide by Salinicoccus spp isolated from Qom Hoze-soltan lake, Iran. 2015;6.

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

20. Chennappa G, Adkar-Purushothama CR, Suraj U, Tamilvendan K, Sreenivasa MY. Pesticide tolerant Azotobacter isolates from paddy growing areas of northern Karnataka, India. World J Microbiol Biotechnol. 2014 Jan 1;30(1):1–7.
21. 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 Sept;28(3):471–9.
22. Elarabi NI, Abdelhadi AA, Ahmed RH, Saleh I, Arif IA, Osman G, et al. *Bacillus aryabhattai* FACU: A promising bacterial strain capable of manipulate the glyphosate herbicide residues. Saudi J Biol Sci. 2020 Sept 1;27(9):2207–14.
23. Shahid M, Khan MohdS. Glyphosate induced toxicity to chickpea plants and stress alleviation by herbicide tolerant phosphate solubilizing Burkholderia cepacia PSBB1 carrying multifarious plant growth promoting activities. 3 Biotech. 2018 Feb 14;8(2):131.
24. Benslama O, Boulahrouf A. High-quality draft genome sequence of *Enterobacter* sp. Bisph2, a glyphosate-degrading bacterium isolated from a sandy soil of Biskra, Algeria. Genomics Data. 2016 June 1;8:61–6.
25. 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 J Agric Sci. 2016;14;38(1):1-3.
26. Jana H, Roy K, Mondal KC. Isolation and characterization of dye degrading bacteria from textile industrial waste, Panskura, West Bengal, India. Indian J Appl Res . 2015 May 1 [cited 2025 Aug 12]; Available from: https://www.semanticscholar.org/paper/Isolation-and-characterization-of-dye-degrading-Jana-Roy/73bb94d10b096d31ed9abc08645cc59c6c6a56fa
27. Rusnam, Gusmanizar N. Characterization of An Acrylamide-degrading Bacterium Isolated from Volcanic Soil. J Environ Bioremediation Toxicol. 2022 Aug 5;5(1):32–7.
28. Sorescu I, Stoica C. Online Advanced Bacterial Identification Software, an Original Tool for Phenotypic Bacterial Identification. Romanian Biotechnol Lett. 2021 Dec 30;26(6):3047–53.
29. 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 July 31;11(1):69–76.
30. 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.
31. Hove-Jensen B, Zechel DL, Jochimsen B. Utilization of Glyphosate as Phosphate Source: Biochemistry and Genetics of Bacterial Carbon-Phosphorus Lyase. Microbiol Mol Biol Rev MMBR. 2014 Mar;78(1):176–97.
32. Zhan H, Feng Y, Fan X, Chen S. Recent advances in glyphosate biodegradation. Appl Microbiol Biotechnol. 2018 June 1;102(12):5033–43.
33. Masotti F, Garavaglia BS, Piazza A, Burdisso P, Altabe S, Gottig N, et al. Bacterial isolates from Argentine Pampas and their ability to degrade glyphosate. Sci Total Environ. 2021 June 20;774:145761.
34. Zhao H, Tao K, Zhu J, Liu S, Gao H, Zhou X. Bioremediation potential of glyphosate-degrading *Pseudomonas* spp. strains isolated from contaminated soil. J Gen Appl Microbiol. 2015;61(5):165–70.
35. Malla MA, Dubey A, Kumar A, Yadav S, Kumari S. Modeling and optimization of chlorpyrifos and glyphosate biodegradation using RSM and ANN: Elucidating their degradation pathways by GC-MS based metabolomics. Ecotoxicol Environ Saf. 2023 Mar 1;252:114628.
36. Sviridov AV, Shushkova TV, Ermakova IT, Ivanova EV, Epiktetov DO, Leontievsky AA. Microbial degradation of glyphosate herbicides (Review). Appl Biochem Microbiol. 2015 Mar 1;51(2):188–95.
37. Wicke D, Schulz LM, Lentes S, Scholz P, Poehlein A, Gibhardt J, et al. Identification of the first glyphosate transporter by genomic adaptation. Environ Microbiol. 2019;21(4):1287–305.
38. Hashem A, Tabassum B, Fathi Abd_Allah E. Bacillus subtilis: A plant-growth promoting rhizobacterium that also impacts biotic stress. Saudi J Biol Sci. 2019 Sept;26(6):1291–7.
39. Su Y, Liu C, Fang H, Zhang D. Bacillus subtilis: a universal cell factory for industry, agriculture, biomaterials and medicine. Microb Cell Factories. 2020 Sept 3;19(1):173.

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).