Arthrobacter sp. GDB: A Glyphosate-Degrading Actinobacterium Isolated from Rhizosphere of Jute (Corchorus olitorius)

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Arthrobacter sp. GDB*:* A Glyphosate-Degrading Actinobacterium Isolated from Rhizosphere of Jute (*Corchorus olitorius*)

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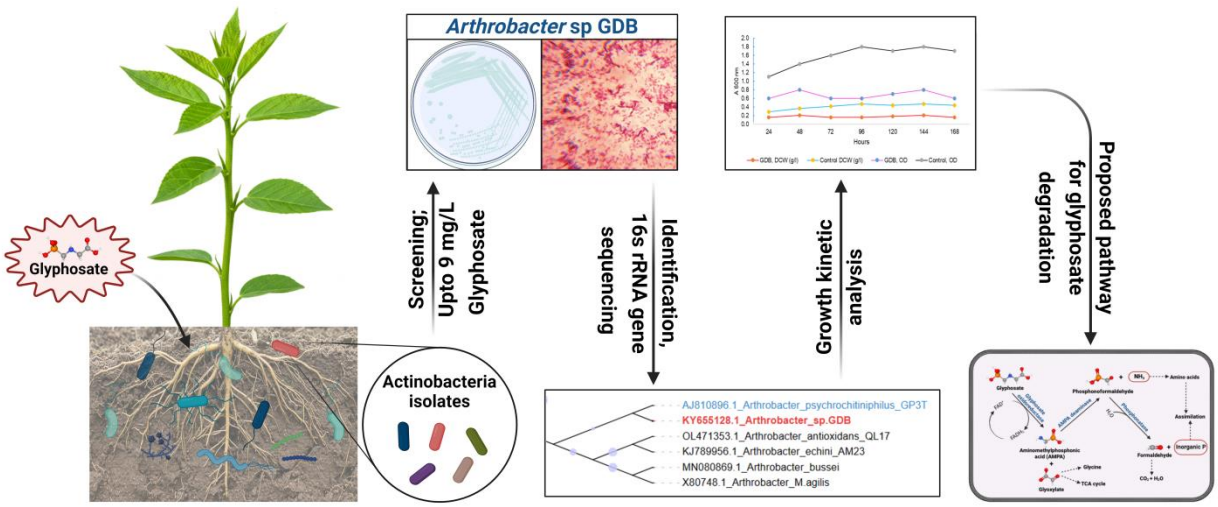
Graphical Abstract



Abstract

A glyphosate-degrading actinobacterial isolate, designated as GDB, was isolated from the rhizosphere soil of jute (*Corchorus olitorius*) cultivated in Barrackpore, India. This Gram-positive, non-motile isolate exhibited rod-coccus morphology and formed creamy white colonies on Luria agar plates. Phylogenetic analysis of its 16S rRNA gene sequence indicated that the GDB isolate belongs to the genus *Arthrobacter*. The isolate demonstrated significant resistance to, and degradation of, glyphosate, thriving in progressively higher herbicide concentrations (3, 6, and 9 mg/L). It reached a maximum optical density of 0.8 and a dry cell weight of 0.208 g/L after 144 hours. Growth kinetic studies showed effective degradation of glyphosate at a concentration of 30 mg/L over time, suggesting the isolate's potential for bioremediation in herbicide-contaminated environments. These findings aim to enhance our understanding of bacterial strategies for herbicide resistance and biodegradation, providing valuable insights for sustainable agricultural practices and environmental safety.

Key Words: *Arthrobacter* sp., Herbicide degradation, Growth kinetics, Biodegradation, Glyphosate.

Introduction

As the global population continues to grow, so does the demand for food, feed, fuel, and fiber. Estimates indicate that by the year 2050, the world will need an astonishing 70% more food to support this population increase (Ghosh et al., 2024). This rising demand highlights the importance of efficient and diverse agricultural production, which encompasses both food and non-food crops.

Jute, often referred to as the "golden fiber," is a major fiber crop that is economically cultivated in countries such as India, China, Bangladesh, Nepal, Indonesia, Myanmar, Brazil, Thailand, and Pakistan. India is the dominant producer of jute goods, providing

approximately 75% of the global output. The average area dedicated to the cultivation of raw jute and mesta is around 662,000 hectares (Information date: Oct 2024, Source: https://www.ibef.org/exports/jute-industry-india). However, controlling weeds is crucial for profitable jute farming. In Bangladesh, around 129 weed species have been identified in jute fields. Many of the dominant weed species found in these fields have also been reported in West Bengal, Assam, and Uttar Pradesh (Dey et al., 2013; Rahman et al., 2012; Singh and Singh, 2014a). Manual weeding is a significant element of jute cultivation, making up about 40% of the total cost. Neglecting this aspect can result in a fiber yield reduction of up to 70% (Ghorai et al., 2008). Weeding alone can account for 30% to 40% or more of the overall cultivation expenses (Islam, 2014). To maximize jute yields, farmers must focus on effective and cost-efficient weed management methods.

While chemical-based weed control strategies contribute to global food security, their reliance can lead to herbicide-tolerant crops, increased cultivation costs, and significant environmental and health issues (Hasanuzzaman et al., 2020). In 2022, global agricultural pesticide consumption reached 3.69 million metric tons, with herbicides accounting for 1.9 million metric tons, while fungicides and bactericides together comprised approximately 793.92 thousand metric tons (Statista, 2024). The extensive use of herbicides has been reported to have harmful impacts on both soil flora and fauna. Rashid et al. (2010) discussed the adverse effects of herbicides on ecosystems, including plants, animals, soil, water quality, aquatic systems, and pest resistance. Herbicide application can also negatively affect soil microbial activity (Sahana et al., 2024). The effects of herbicides on earthworms are so significant that they are commonly used as biological indicators to monitor herbicide contamination in soil (Kumar et al., 2022). Metribuzin herbicides, in particular, cause soil and water contamination and can lead to both acute intoxication and chronic health effects (Samir et al., 2020).

Commonly used herbicides worldwide include glyphosate, atrazine, paraquat, 2,4-dichlorophenoxyacetic acid (2,4-D), diuron, pendimethalin, imazapyr, and metolachlor, all of which pose threats to both terrestrial and aquatic organisms, even at low concentrations (Marin-Morales et al., 2013). Glyphosate is widely utilized globally as a non-selective herbicide for controlling various weeds in numerous industries and cropping systems (Anarghou et al., 2024). However, glyphosate's persistence in soil, due to its amphoteric nature, non-volatility, and high adsorption, makes it a significant environmental contaminant (Ogunbiyi et al., 2023).

Plant growth-promoting rhizobacteria (PGPR) play a critical role in maintaining soil health and plant productivity (Rani et al., 2022). An alternative approach to mitigate the effects of herbicidal toxicity is through bioremediation, which exploits the ability of such microorganisms to degrade harmful substances into less toxic forms. The active biodegradation of herbicides in soil and water by different bacterial strains has been documented (Chandrasekaran and Paramasivan, 2024; Singh and Singh, 2014b). Roy et al. (2015) suggested that in jute fields, *Trichoderma harzianum* and *Pseudomonas fluorescens* significantly contribute to the biodegradation of herbicides.

On a specific note, the genus *Arthrobacter* is a Gram-positive actinobacterium commonly found in soils and environments contaminated with chemicals and heavy metals. This adaptability is due to its efficiency in nutrient use and its tolerance to various environmental stressors. Clark and Wright (1970) were the first to report that soil actinobacteria, specifically Arthrobacter and Achromobacter, can metabolize isopropyl N-phenyl carbamate (IPC) and use it as their sole carbon source. *Arthrobacter aurescens* strain TC1, isolated from soil, has been shown to degrade s-triazine-based herbicides (Strong et al., 2002). This strain can utilize atrazine as its only source of carbon and nitrogen, capable of transforming 3,000 mg of atrazine per liter in a growing culture. Additionally, Wang and Xie (2012) identified a highly efficient atrazine-degrading soil bacterium, *Arthrobacter* sp. DAT1, which exhibits a broad

optimum pH and temperature range. This strain can utilize atrazine as its sole nitrogen source and carries herbicide-resistant genes, including atzB, atzC, and trzN.

Despite the increasing demand for sustainable agricultural practices, weed management in jute cultivation still heavily relies on chemical herbicides. This reliance leads to environmental contamination, soil degradation, and microbial imbalance. The excessive use of herbicides such as glyphosate has raised concerns due to its persistence in soil, negative effects on non-target organisms, and potential health risks for humans. While bioremediation using plant growth-promoting rhizobacteria (PGPR) has shown promise in reducing herbicide toxicity, there is limited research on the role of actinobacteria, particularly *Arthrobacter* species, in degrading glyphosate in jute fields. Previous studies on microbial herbicide degradation have primarily focused on model crops, thus leaving a gap in understanding how native soil microbes in jute cultivation systems contribute to glyphosate detoxification.

To address this gap, the present study aims to isolate and characterize a glyphosate-degrading actinobacterium from the rhizosphere of jute crops cultivated in India. The study will specifically focus on identifying *Arthrobacter* species and evaluating their potential to degrade high concentrations of glyphosate. By exploring the herbicide degradation capabilities of indigenous microbial strains, this research seeks to provide an eco-friendly alternative for mitigating glyphosate contamination and promoting sustainable jute farming.

Materials and Methods

Media and Growth Conditions:

Actinomyces Isolation Agar (AIA) was used for isolating actinomycetes for further characterization. The AIA composition included sodium caseinate (2.000 g), L-asparagine (0.100 g), sodium propionate (4.000 g), dipotassium phosphate (0.500 g), magnesium sulfate (0.100 g), ferrous sulfate (0.001 g), and agar (15.00 g/L), sourced from Himedia

Laboratories, India. All cultures were maintained in test tubes containing 10 mL of AIA medium after incubation at 28 °C and were subsequently stored at 4 °C in a refrigerator.

Sampling Site and Characterization of Rhizosphere Soils:

Samples of jute rhizosphere soil were collected from the ICAR-CRIJAF site in Barrackpore, located in the 24 Parganas (North) district of India. This site is situated approximately at 22°45'16.53" N latitude and 88°25'19.25" E longitude, with an elevation of 9.69 m above mean sea level. The predominant soil type in the area ranges from sandy to clay sandy loam, with a high:medium:low land ratio of 17:33:39. The pH of the surface soil is nearly neutral, ranging between 6.5 and 7.5, while the subsoil tends to be acidic. Generally, the soils are deficient in available nitrogen (14.0 to 17.8 mg N/100 g), but they have a medium organic carbon content (0.53 to 0.69% C) and are rich in available phosphorus (8.1 to 12.4 mg P2O5/100 g).

Experimental Setup for Collection of Soil Samples from the Jute Rhizosphere:

Soil samples were collected within six hours from the jute rhizosphere that had been exposed to the herbicide treatments: glyphosate (3 mL/L); glyphosate (3 mL/L) + paraquat (2 mL/L); paraquat (6 mL/L); and glyphosate (5 mL/L) + paraquat (2 mL/L). Because jute is a tall crop, traditional pots were impractical, leading to the use of drums. Each drum measured 584 mm in diameter and 876 mm in height. A total of 16 drums were utilized, with each herbicide treatment combination randomized and replicated four times under controlled cultural conditions.

Isolation and Selection of Herbicide-Resistant Actinobacteria:

One gram of soil was collected from the rhizosphere of jute plants grown in the experimental drums. The serial dilution technique (10^-9) using sterile distilled water was employed for bacterial isolation. The ninth dilutions were spread onto AIA medium supplemented with the

158 experimental herbicide combinations from the drums, followed by incubation at 28 °C for
159 seven days. After incubation, the isolates were subjected to repeated exposure every seven
160 days over two months under various herbicide conditions, including glyphosate (3 mL/L),
161 glyphosate (3 mL/L) + paraquat (2 mL/L), paraquat (6 mL/L), and glyphosate (5 mL/L) +
162 paraquat (2 mL/L). Actinobacteria demonstrating resistance was screened for an additional
163 six days under double concentrations of these herbicides. Following this, cultural
164 characteristics like colony morphology and Gram staining reactions were performed to
165 assess bacterial survival and analyze their morphology, confirming the presence of
166 actinomycetes. Actinobacteria isolated from the second round of screening after fifty days
167 were further tested for herbicide resistance on media with triple concentrations of the
168 herbicides (glyphosate 9 mL/L; 9 mL/L glyphosate + paraquat 6 mL/L; paraquat 6 mL/L;
169 glyphosate 15 mL/L + paraquat 6 mL/L) in a third screening. Notably, the third round of
170 screening revealed that glyphosate (9 mL/L) promoted the growth of glyphosate-degrading
171 bacterial actinomycetes, designated as GDB. The GDB isolate was subsequently selected
172 for kinetic studies.

173 *Growth Kinetics and Resistance Patterns of the Herbicide-Resistant Isolates:*

174 Actinobacterium cells collected from the third screening were washed three times with
175 phosphate buffer (pH 7.0) under vigorous shaking (250 rpm for 1 hour) to ensure thorough
176 cleansing. Degradation efficiency was assessed in Actinomyces Isolation Broth (AIB) by
177 growing the isolate GDB while shaking at 250 rpm at 32 °C, with optical density (OD)
178 recorded spectrophotometrically at 24-hour intervals. The OD measurements at 660 nm
179 quantified cell density, with 1 OD corresponding to 0.26 g dry cell weight per liter (Shi et al.,
180 2014). The degradation efficacy of isolate GDB was tested in glyphosate at a concentration
181 of 30 mL/L. Controls were established for each experiment to ensure accurate results.
182 Glyphosate degradation was monitored by measuring absorbance at specific intervals using
183 a spectrophotometer (CECIL INSTRUMENTS, India). Absorbance readings were taken at

24, 48, 72, 96, 120, 144, and 168 hours over a period of seven days. This method aligns with the 24-hour interval approach used by Ma et al. (2009) for herbicide degradation measurement. Observations recorded at each time point were plotted against absorbance to determine growth kinetics and resistance patterns by counting dry cell weight.

DNA Extraction, PCR, and Phylogenetic Tree Construction:

In this study, genomic DNA extraction from actinomycete samples was performed using the DNeasy® Plant Mini Kit (QIAGEN, Germany), following the manufacturer's protocol. The extracted DNA was quantified using a spectrophotometer (CECIL INSTRUMENTS, India).

PCR amplification targeted the 16S rRNA gene region using the primers F243 and R513GC (Heuer et al., 1997). Each 25 µL PCR reaction mixture contained 2.5 µL of 1x buffer (Fermentas Canada Inc.), 0.5 µL of dNTPs (200 µM each), 0.5 µL of each primer F243 and R513GC (Genuine Chemical Corporation, India; at a concentration of 0.1 µM each), 1.5 µL of MgCl2 (25 mM), 0.5 µL of Taq DNA polymerase (2.5 U), 2 µL of DNA (100 ng), and 17 µL of sterile distilled water. The PCR was conducted in a MyCycler™ thermal cycler (Biorad, USA), starting with an initial denaturation at 94 ºC for 2 minutes, followed by 30 cycles of: 94 ºC for 1 minute (denaturation), 55 ºC for 1 minute (annealing), and 72 ºC for 2 minutes (elongation), with a final elongation at 72 ºC for 10 minutes. The amplified fragments F243 and R513GC were analyzed by gel electrophoresis in 1.4% agarose gels prepared with 1x TBE buffer (Sambrook and Russell, 2001), stained with ethidium bromide, and visualized under UV light. The PCR products were subsequently sequenced by Chromous Biotech Pvt. Ltd., India (with information on GenBank submission represented by the reference strain Arthrobacter sp. GDB, KY655128.1). Sequence identification was performed using the BLAST search tool (Altschul et al., 1990) to compare the obtained sequences with those in the NCBI database based on the highest BLAST scores.

A comprehensive phylogenetic analysis was conducted to determine the evolutionary relationships. The analysis began with the partial 16S ribosomal RNA gene sequence of the *Arthrobacter* sp. isolate GDB, which was used as a query for a BLAST search against the EzTaxon database. To expand the evolutionary context and potentially include entries missing from GenBank, the curated 16S rRNA database of EzTaxon was utilized. The query sequence was then used in a BLAST search against all 96 *Arthrobacter* species available in EzTaxon, retrieving 26 *Arthrobacter* species with moderately high sequence identity (>95%). These 26 closest relatives identified from EzTaxon, along with our target sequence, were used to create a dataset of 27 sequences for phylogenetic analysis. The sequences were aligned using the Multiple Sequence Comparison by Log-Expectation (MUSCLE) algorithm, and a phylogenetic tree was constructed using the neighbour-joining (NJ) method within MEGA 11 software. The constructed phylogenetic tree was then visualized using iTOL.

Results

Isolation and Selection of Actinobacteria:

In this experiment, we found that the rhizosphere of jute contained bacteria at a density of 4.1×10^5 colony-forming units (CFU) per gram of soil, actinobacteria at 1.56×10^4 CFU/g, and fungi at 1.81×10^3 CFU/g. This indicates that bacteria were the most abundant, followed by actinobacteria and fungi in the rhizosphere soil. The microbial communities exhibited diversity based on their cultural characteristics.

The actinobacteria were screened for their ability to resist herbicides at various concentrations, demonstrating differential resistance among strains to the tested combinations of herbicides. Soil samples were collected from herbicide-treated drums containing jute rhizosphere soil. The treatments included glyphosate (3 mL/L), glyphosate (3 mL/L) + paraquat (2 mL/L), paraquat (2 mL/L), and glyphosate (5 mL/L) + paraquat (2 mL/L). In our study, we identified and characterized actinobacterial isolates that were resistant to

glyphosate. Initially, one isolate showed resistance at a glyphosate concentration of 3 mL/L. Subsequent screenings, which involved doubling the herbicide concentration, revealed that only one isolate maintained resistance at 6 mL/L. In the final screening phase, this isolate also demonstrated resistance at 9 mL/L.

We monitored the growth and resistance patterns of the actinobacterial isolates in both plain and herbicide-containing media. The results indicated that under control conditions, two isolates were found initially, then only one later. With glyphosate, one similar isolate was obtained at increasing concentrations: 3 mL/L, 6 mL/L, and 9 mL/L (see Table 1).

Table 1 Screening resistance levels of five actinobacterial isolates in three concentrations of glyphosate.

	Number of isolates survived in the		
Herbicide Treatments	1st Screening phase (3 mL/L)	2nd Screening phase (6 mL/L)	3rd Screening phase (9 mL/L)
Control	2	2	1
Glyphosate	1	1	1

Cultural Characteristics of the Actinobacterium:

After the first and second screenings, the colonies appeared whitish-gray, which indicates their general appearance. They exhibited no pigmentation, suggesting that they do not produce colored metabolites under the given growth conditions. These non-translucent colonies were opaque. The actinomycete GDB, which is resistant to glyphosate (6 ml/L), displayed these characteristics (Fig. 1a). The cells of the actinobacterium GDB were Gram-positive, non-motile, and exhibited a rod-coccus morphology (Fig. 1b).

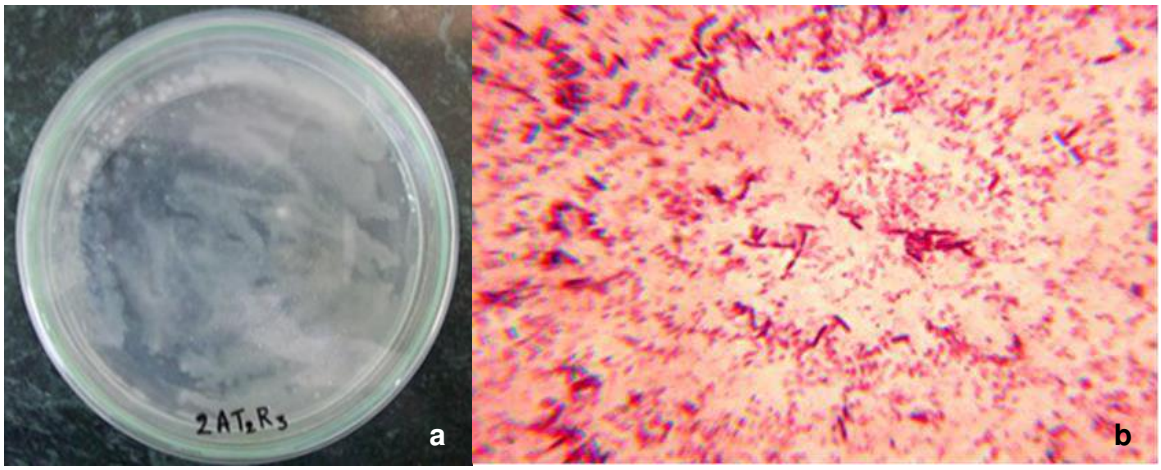


Fig.1. (a) Colony morphology of the glyphosate-resistant actinomycete isolate and (b) Gram-stained actinobacterium strain GDB).

Growth Kinetics:

During the third screening, the GDB isolate underwent detailed growth kinetic analysis through spectrophotometric observations. When grown with 30 mL/L glyphosate, the GDB isolate exhibited an initial optical density (OD) of 0.6 after 24 hours, increasing to 0.8 at 48 hours. However, by 72 and 96 hours, the OD decreased back to 0.6. A slight increase to 0.7 was observed at 120 hours, followed by another rise to 0.8 at 144 hours, and a decline to 0.6 at 168 hours. The corresponding dry cell weights for the GDB isolate ranged from 0.156 g/L to 0.208 g/L (refer to Table 2 and Fig. 2).

Throughout the 24 to 168-hour monitoring period, the OD of GDB revealed distinct growth phases: an initial lag phase (0-24 hours), a log phase (24-48 hours), a stationary phase (72-96 hours), a second lag phase (120-144 hours), another log phase (144-168 hours), and ultimately a decline phase. In contrast, the control group demonstrated consistent growth, with ODs of 1.1, 1.4, 1.6, 1.8, 1.7, 1.8, and 1.7 over the 7-day observation period. The corresponding dry cell weights for the controls ranged from 0.286 g/L to 0.468 g/L.

Table 2. Average optical densities (OD) at 660 nm and the corresponding dry cell weight (DCW) (g/L) for the GDB strain with herbicides over a period of 7 days.

Hours	Control OD	Control DCW (g/L)	GDB, OD	GDB DCW (g/L)
24	1.1	0.286	0.6	0.156
48	1.4	0.364	0.8	0.208
72	1.6	0.416	0.6	0.156
96	1.8	0.468	0.6	0.156
120	1.7	0.442	0.7	0.182
144	1.8	0.468	0.8	0.208
168	1.7	0.442	0.6	0.156

Footnote: GDB strain was cultivated with 30 mL/L of glyphosate, and a control group without glyphosate was included for comparison. Measurements were taken at 24-hour intervals.

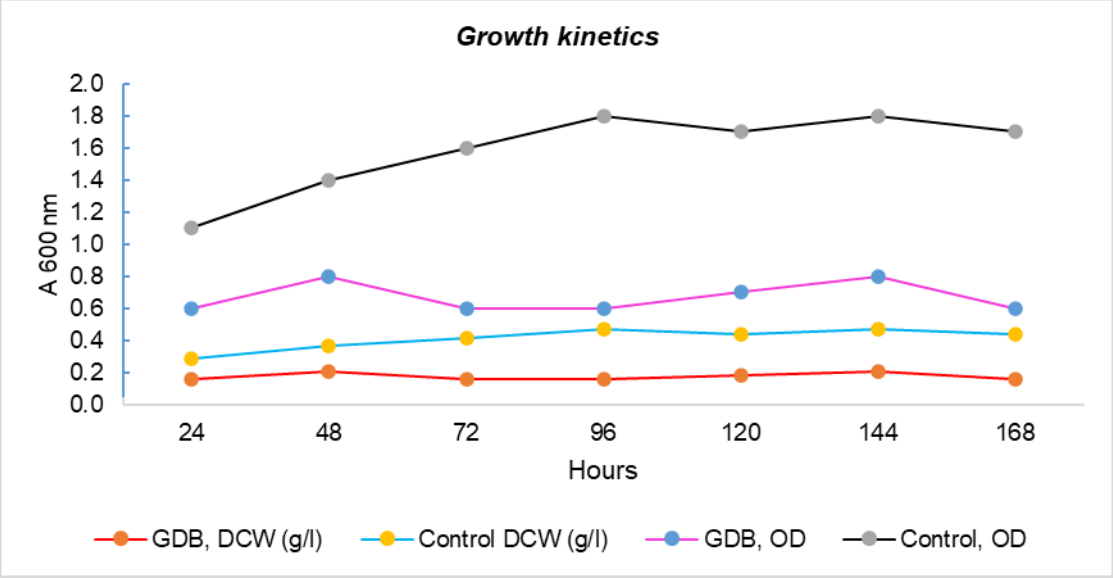


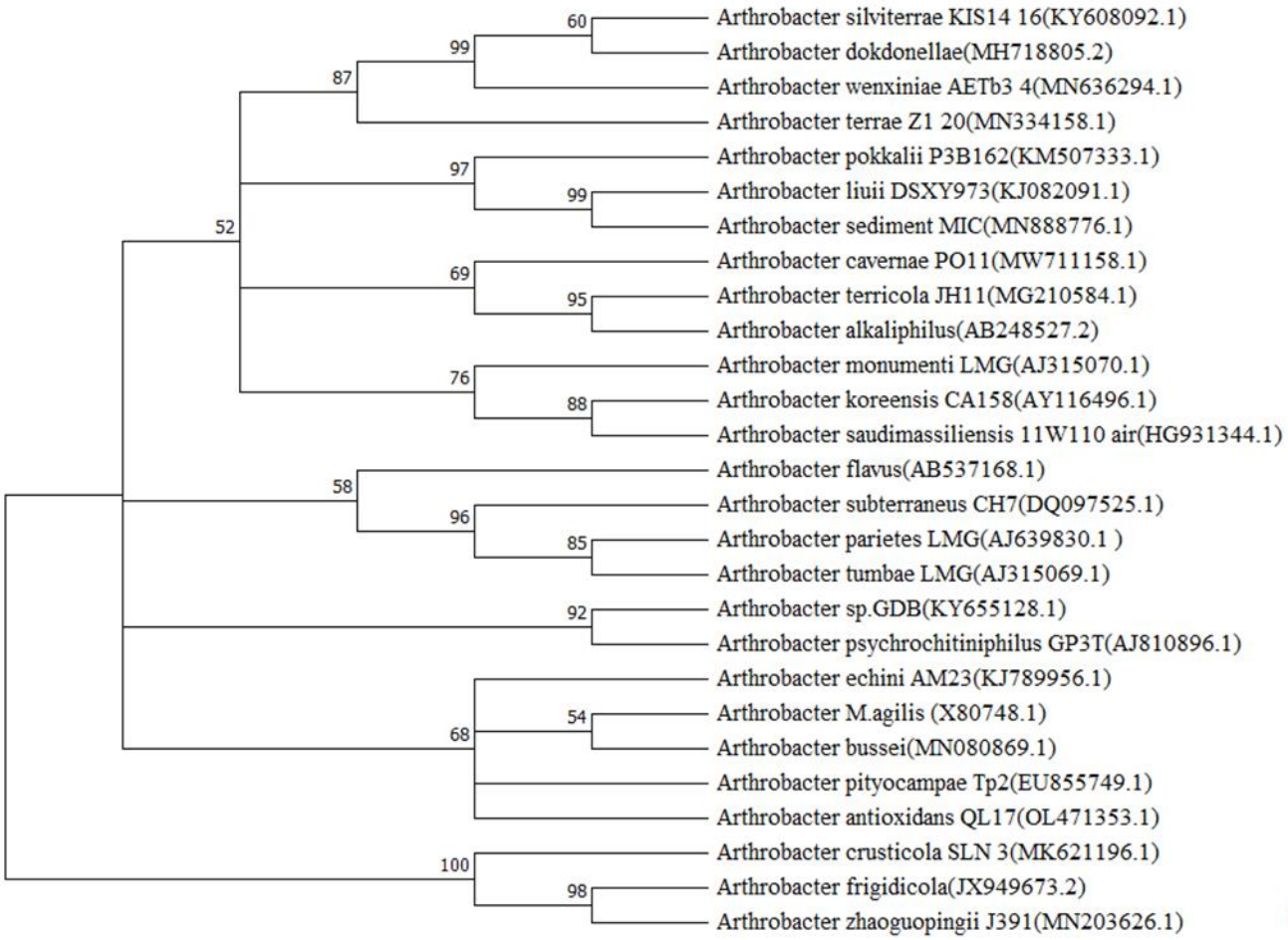
Fig. 2. The growth kinetics (optical density) and degradation efficiency (dry cell weight) of GDB under conditions amended with glyphosate (30 mL/L) compared to control conditions over a 7-day period. Absorbance at 660 nm and dry cell weight were recorded at 24-hour intervals, from 24 hours to 168 hours.

Phylogenetic Analysis:

A phylogenetic analysis using 16S rRNA sequences from 27 *Arthrobacter* species provided valuable insights into the relationships within the genus *Arthrobacter*. The resulting phylogenetic tree revealed two major groups, which were further divided into four subgroups, offering a comprehensive view of their evolutionary relationships.

In the primary major group, a novel *Arthrobacter* species designated *Arthrobacter* sp. GDB (NCBI Accession KY655128.1), was found on a separate branch distinct from all previously known Arthrobacter species (Fig. 3). Notably, *Arthrobacter* sp. GDB formed a unique cluster within this subgroup alongside only one other species, *Arthrobacter psychrochitiniphilus*, with a bootstrap value of 92%. This unique branch, supported by high statistical confidence, indicates that *Arthrobacter* sp. GDB has sufficient genetic divergence from other *Arthrobacter* species to be classified as a novel species.

Other well-known *Arthrobacter* species, such as *Arthrobacter pityocampae*, *A. bussei*, and *A. koreensis*, were also included in this major group but clustered differently in the phylogenetic tree. The second major group comprises only three species: *Arthrobacter crusticola*, *A. frigidicola*, and *A. zhaoguopingii*. These species show significant divergence from *Arthrobacter* sp. GDB, indicating their evolutionary distance from this novel species.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Arthrobacter silviterrae KIS14 16(KY608092.1)
Arthrobacter dokdonellae(MH718805.2)
Arthrobacter wenxiniae AETb3 4(MN636294.1)
Arthrobacter terrae Z1 20(MN334158.1)
Arthrobacter pokkalii P3B162(KM507333.1)
Arthrobacter liuii DSXY973(KJ082091.1)
Arthrobacter sediment MIC(MN888776.1)
Arthrobacter cavernae PO11(MW711158.1)
Arthrobacter terricola JH11(MG210584.1)
Arthrobacter alkaliphilus(AB248527.2)
Arthrobacter monumenti LMG(AJ315070.1)
Arthrobacter koreensis CA158(AY116496.1)
Arthrobacter saudimassiliensis 11W110 air(HG931344.1)
Arthrobacter flavus(AB537168.1)
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Arthrobacter echini AM23(KJ789956.1)
Arthrobacter M.agilis (X80748.1)
Arthrobacter bussei(MN080869.1)
Arthrobacter pityocampae Tp2(EU855749.1)
Arthrobacter antioxidans QL17(OL471353.1)
Arthrobacter crusticola SLN 3(MK621196.1)
Arthrobacter frigidicola(JX949673.2)
Arthrobacter zhaoguopingii J391(MN203626.1)

a

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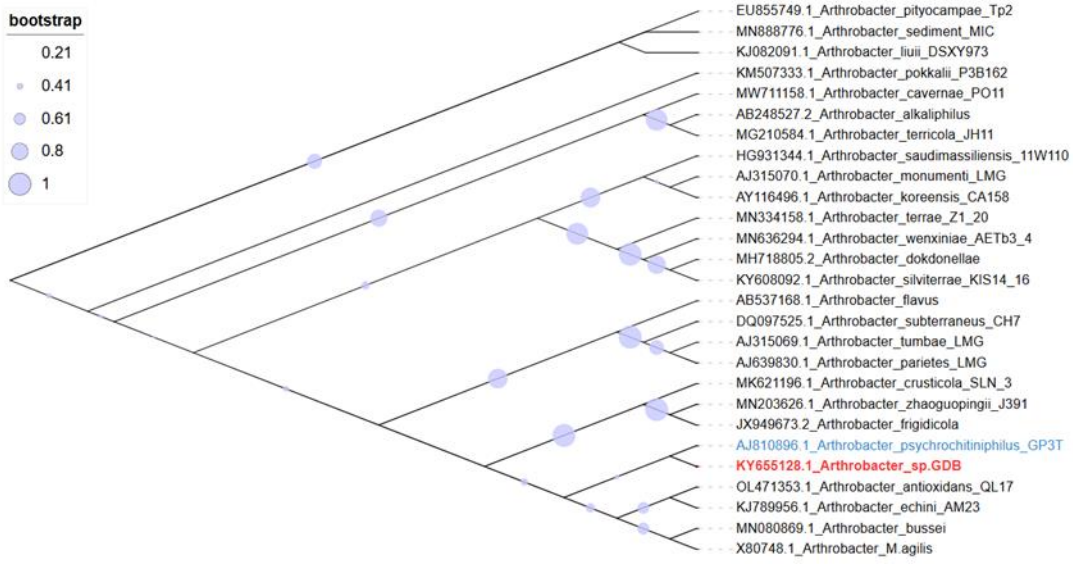
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KJ789956.1_Arthrobacter_echini_AM23
MN080869.1_Arthrobacter_bussei
X80748.1_Arthrobacter_M.agilis

b

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Fig. 3. The phylogenetic analysis of *Arthrobacter* sp. GDB was conducted using 16S rRNA sequences from 26 different species of *Arthrobacter*. The results from both the neighbor-joining (a) and maximum likelihood (b) trees revealed two major groups and four subgroups within the genus. Strain GDB forms a distinct branch alongside *A. psychrochitiniphilus*, demonstrating a high bootstrap value. This finding emphasizes its unique evolutionary lineage within the largest major group, supporting its distinctiveness.

Discussion

The rhizosphere soil is known to harbor a diverse array of microbial species and populations (Olahan et al., 2016). Previous research has documented the microbial richness of the rhizosphere. For instance, Majumdar (2017) reported total microbial populations (including bacteria, fungi, and actinomycetes) ranging from 8×10^6 to 22×10^7 CFU per gram of soil. In our study, we quantified actinomycetes within the jute rhizosphere, revealing a population of 1.56×10^4 CFU per gram of soil. This finding confirms that actinobacteria are an abundant microbial group in the soil.

Notably, actinobacteria play a significant role in herbicide degradation (Kim et al., 2024). Elsayed et al. (2022) isolated 45 actinobacterial strains from jute rhizosphere soil. Additionally, studies on related soil types, such as rice soil, have identified specific degradative bacteria. For example, Zhang et al. (2010) isolated microorganisms from rice soil, which shares similar characteristics with jute crop soil, and identified *Pseudomonas putida* 4CD1. This strain was shown to degrade p-coumaric acid and other phenolic compounds, achieving 70-93% degradation in inorganic solutions and 99% in Luria-Bertani broth within 48 hours. Recent research has also highlighted the ability of various bacterial strains to degrade herbicides like glyphosate. Mohy-Ud-Din et al. (2024) reported that *Serratia liquefaciens* WAG2, *Klebsiella variicola* WAG4, *Enterobacter cloacae* WAG45, *Pseudomonas aeruginosa* WAG9, and *Enterobacter ludwigii* WAG11 significantly reduced glyphosate concentrations in spiked liquid media and soil. Actinomycetes are also known to

degrade herbicides such as alachlor, aldrin, carbofuran, chlorpyrifos, and diuron in soil and water through the action of extracellular enzymes, contributing to bioremediation (Datta et al., 2024; Sette et al., 2005).

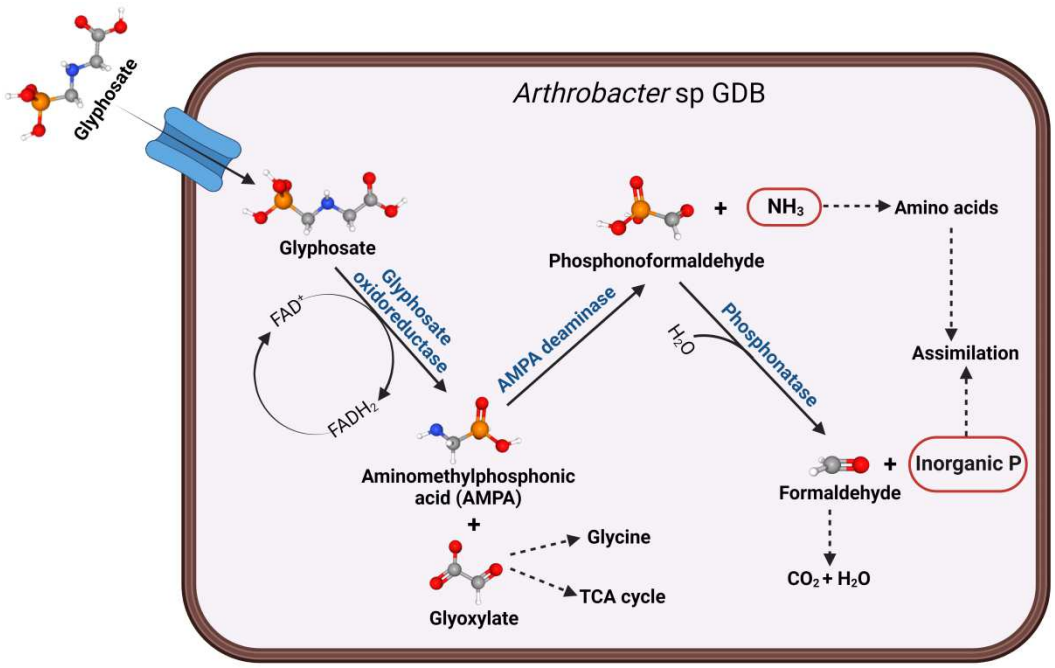
Building on this knowledge, our study specifically assessed the degrading capacity of actinobacteria from the jute rhizosphere using glyphosate, 2,4-D, and paraquat. We conducted a three-phase screening for glyphosate resistance in these actinobacterial isolates. One isolate, designated GDB, consistently exhibited resistance, tolerating increasing glyphosate concentrations. This suggests its effective glyphosate degradation capability and maintenance of functionality even under high concentrations. Furthermore, there is currently no widely available and suitable method for in situ glyphosate residue detection. Therefore, identifying novel microorganisms capable of efficient glyphosate degradation, such as GDB, is crucial for mitigating glyphosate contamination.

The dry cell weight (DCW), represented by optical density (OD) values is crucial for understanding microbial growth and biomass production. It provides a quantitative measure of the microbial population's size and metabolic activity. The experimental results showed a maximum DCW of 0.208 g/L observed after 48 and 144 hours, respectively, indicating that strain GDB achieved peak biomass after two distinct time periods. This information is important for optimizing conditions for glyphosate degradation, improving bioremediation strategies, and understanding the metabolic capabilities of GDB in comparison to other microbial cultures.

The experiment revealed that strain GDB achieved a maximum OD of 0.8 during both the first and second log phases of growth. This indicates that GDB effectively utilized glyphosate as a phosphorus source during these stages. Consistently, our experiment demonstrated that glyphosate likely served as the sole phosphorus source for GDB, suggesting a possible degradation pathway. Microorganisms can utilize glyphosate as a primary phosphorus source due to its structural similarity to phosphoenolpyruvate (PEP), a crucial compound in

the shikimate pathway (Duke and Powles, 2008). This suggests a potential degradation pathway primarily involving C-N bond cleavage, resulting in the formation of aminomethylphosphonic acid (AMPA). Similar findings have been reported, where oxidase-mediated C-N bond cleavage produces AMPA and glyoxylate (Sviridov et al., 2015). However, GDB's unique ability to utilize glyphosate twice for degradation suggests a different metabolic behavior compared to the mixed culture of *Pseudomonas* sp. and *Bacillus* sp. reported by Wijekoon and Yapa (2018), who observed a steady increase in glyphosate degradation up to 72 hours, followed by a gradual decrease up to 120 hours. This difference highlights the distinct metabolic characteristics of GDB.

While glyphosate degradation is primarily regulated by the AMPA pathway, an alternative sarcosine pathway also exists (Pipke and Amrhein, 1988; Zhan et al., 2018; Singh et al., 2019). In this sarcosine pathway, microorganisms use enzymatic mechanisms, such as direct carbon-phosphorus (C–P) bond cleavage by C–P lyase, resulting in the production of sarcosine and inorganic forms of carbon and phosphorus (Sviridov et al., 2015). *Arthrobacter* sp. strain GLP-1 has been reported to degrade glyphosate via the sarcosine pathway. However, this strain applied glyphosate as a carbon source when it was cross-contaminated with *Klebsiella pneumoniae* (Pipke et al., 1987). This indicates that, in the absence of phosphorus, likely consumed by *K. pneumonia*, *Arthrobacter* could also utilize carbon as an energy source. In our study, no cross-contamination with other microbes occurred. Hence, our results support the conclusion that *Arthrobacter* sp. strain GDB utilized glyphosate as a phosphorus source through the AMPA pathway (Fig. 4).



368

369 **Fig. 4.** A proposed enzymatic pathway for the biodegradation of glyphosate in *Arthrobacter*
370 sp. strain GDB, which operates effectively in the presence of aminomethylphosphonic acid
371 (AMPA). This highlights the potential of this strain for bioremediation efforts.

372 In the AMPA pathway, many microorganisms use glyphosate as their primary source of
373 phosphorus. Some microbes can further degrade AMPA, converting its carbon to carbon
374 dioxide, even if they do not utilize the phosphorus released (Pipke et al., 1987). Certain
375 bacterial strains, such as the mutant *Arthrobacter* sp. GLP1/Nit, utilize glyphosate as a
376 nitrogen source (Pipke and Amrhein, 1988). Additionally, *Arthrobacter* sp. AD26 can use
377 other herbicides, like atrazine, as a nitrogen source (Li et al., 2008).

378 Pollegioni et al. (2011) reported that glyphosate biodegradation in *Arthrobacter* involves the
379 uptake of glyphosate into the cell. Glyphosate oxidoreductase converts glyphosate to AMPA
380 and glyoxylate. AMPA is then degraded by AMPA deaminase into phosphonoformaldehyde
381 and ammonia (Zboińska et al., 1992). Phosphonoformaldehyde can further be converted to
382 formaldehyde and phosphate by phosphonoformaldehyde hydrolase or phosphonatase.
383 Glyoxylate enters central metabolic pathways, leading to the formation of glycine or
384 integration into the tricarboxylic acid (TCA) cycle. Finally, formaldehyde is oxidized to CO_2,

phosphate is assimilated, and ammonia is integrated into amino acids (Guardado-Fierros et al., 2025).

In our experiment, we prepared three herbicide compositions containing paraquat; however, we did not observe any growth of actinomycetes under the test conditions. Previous studies primarily documented paraquat degradation by microorganisms, especially fungi. For instance, *Aspergillus tamarii* degraded paraquat by 80%, and *Cunninghamella* sp. degraded it by 63% in soil samples from northern Thailand contaminated with paraquat (Wongputtisin et al., 2021). However, Ataikiru and Okorhi (2022) reported contrasting results, noting complete paraquat degradation (99.7%) by soil microorganisms, including *Bacillus*, *Pseudomonas*, *Flavobacterium*, *Arthrobacter*, and *Sphingomonas*, at recommended field rates. Degradation dropped to 46.3% when the treatment rate was increased to four times the recommended amount. Their study also included carbofuran insecticides, suggesting that the additional energy source may have influenced paraquat degradation.

Through phylogenetic analysis, we aimed to gain comprehensive insights into the genetic relationships within the genus *Arthrobacter*, highlighting the unique position of *Arthrobacter* sp. GDB. The strain GDB's robust herbicide-degrading capabilities indicate its potential environmental significance. The phylogenetic tree, constructed using 26 16S rRNA sequences from *Arthrobacter*, revealed two major groups, providing a framework for understanding the evolutionary relationships within this genus. The distinct branch with high bootstrap support reinforces the novelty of GDB (López-Jiménez et al., 2023).

Arthrobacter sp. GDB clusters closely with *Arthrobacter psychrochitiniphilus* in the phylogenetic tree, which is supported by a high bootstrap value. This close clustering likely reflects shared ancestry and further emphasizes the uniqueness of GDB. Despite their phylogenetic proximity, *Arthrobacter* sp. GDB inhabits a distinct ecological niche compared to *Arthrobacter psychrochitiniphilus*, which aligns with the concept of niche partitioning (Giraldo-Kalil et al., 2023). It is noteworthy that we isolated *Arthrobacter* sp. GDB from a hot

climate, while *Arthrobacter psychrochitiniphilus* was initially isolated from Antarctica (Wang et al., 2009). This ecological differentiation, combined with its distinct phylogenetic position, suggests that *Arthrobacter* sp. GDB represents a novel species equipped with significant adaptations to its thermal environment.

Further phylogenetic analysis indicates that *Arthrobacter* sp. GDB has a unique ability to degrade high concentrations of herbicides, such as glyphosate and 2, 4-D. This capability, along with its distinct genetic profile, supports its classification as a novel species and highlights its potential environmental importance as an herbicide-degrading actinobacterium. Although primarily designed for metagenomic analysis, the EzTaxon curated database provided a valuable resource to complement the highly similar sequences identified in NCBI GenBank. By analyzing the position of *Arthrobacter* sp. GDB within the constructed tree relative to closely related species, particularly focusing on distinct and well-supported branches, we aim to evaluate its status as a novel species.

Our research identified *Arthrobacter* sp. GDB as highly valuable for sustainable weed management practices. This strain enhances bioremediation technologies by effectively removing herbicide contamination. Similarly, Li et al. (2008) reported that *Arthrobacter* sp. strain AD26 efficiently degrades atrazine, reducing atrazine levels in soil by 98% within 20 days at 26 °C, making it a strong candidate for bioremediation. While actinomycetes are known for their ability to degrade a variety of herbicides, the specific capability of *Arthrobacter* sp. GDB to target glyphosate suggests that further research is necessary to explore its potential for degrading other herbicides and optimizing conditions for broader degradation. Phylogenetic analysis has established the uniqueness and distinct genetic positioning of *Arthrobacter* sp. GDB. However, conducting whole-genome sequencing and transcriptomic analyses would provide deeper insights into the metabolic pathways involved in glyphosate degradation.

To better understand GDB's capabilities, it would be beneficial to compare its degradation rates, enzyme activities, and metabolic efficiency directly with those of other known glyphosate-degrading strains, such as *Pseudomonas* sp., *Bacillus* sp., or other species of *Arthrobacter*. GDB's ability to break down glyphosate indicates that it may be specifically adapted to environments rich in this herbicide. Nonetheless, future studies should evaluate its survivability and metabolic stability across various soil types and environmental conditions to assess its broader applicability. Furthermore, research into formulation development, microbial viability, and delivery methods (such as bio-fertilizers or seed coatings) is crucial for translating these findings into practical applications. By focusing on genomic studies, field trials, comparative analyses, and formulation strategies, we can strengthen the case for *Arthrobacter* sp. GDB as a promising bioremediation agent for sustainable agriculture and the restoration of soils contaminated with herbicides.

Conclusion

The global agricultural sector is grappling with significant challenges due to the overuse of pesticides, including herbicides, insecticides, fungicides, and bactericides. However, our findings highlight the promising role of soil microorganisms in the degradation of herbicides. In our pure culture experiments, GDB actinomycete isolates have shown remarkable effectiveness in metabolizing such harmful substances. Particularly impressive is the Arthrobacter sp. strain GDB, isolated from the rhizosphere of jute plants, which holds great potential for bioremediation by breaking down high concentrations of glyphosate.

This strain demonstrates a remarkable ability to resist glyphosate concentrations of up to 9 mL/L, outperforming other isolates in terms of resistance. Our quantitative results indicate that the maximum optical density (OD) of the glyphosate-resistant strain GDB reached 0.8, while the dry cell weight (DCW) ranged from 0.156 g/L to 0.208 g/L over 7 days when exposed to 30 mL/L of glyphosate. These findings suggest that microorganisms from the jute rhizosphere could serve as a valuable resource for isolating herbicide-resistant genes. To

fully unlock their potential, further research is essential to explore the genetic basis of herbicide resistance and degradation pathways. This includes ongoing efforts in identifying genes and characterizing their molecular functions. Together, these initiatives can lead to a more sustainable future in agriculture.

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