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Isolation, Characterization and Growth Optimization of Glufosunate Ammonium Degrading Bacteria from Farmlands Soil in Mubi

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Abstract

The persistent application of glufosinate ammonium as a non-selective herbicide in agricultural practices raises significant environmental concerns due to its potential accumulation and eco-toxicity. This study focused on the isolation, characterization, degradation and optimization of glufosinate ammonium degrading bacteria from agricultural soil in Mubi, Adamawa State, Nigeria. Three isolates were screened from mineral salt medium containing glufosinate ammonium herbicides as carbon source which are isolate GA1, GA2 and GA3 but the isolate GA3 strain show highest degradation potential of glufosinate ammonium compare to GA1 and GA2 which was determined by UV-spectrophotometer and further by LC-MS which showed degradation by 75.9% the GA3 strain was characterized morphologically and biochemically as gram positive, and through 16SrRNA gene sequencing and phylogenetic analysis confirmed isolate GA3 (94.45% 16SrRNA homology to Bacillus). Optimization of the degradation condition was performed using the one factor at a time (OFAT) approach, which revealed that the strain has growth optima at 35^{o}C, pH 7.0, 10 mg/L glufosinate ammonium, with 5% inoculum size, and 48 hours' incubation peak growth, the GA3 stain had growth stimulated best with glutamic acid 1g/L, (nitrogen source), and sucrose 1g/L (carbon source), exposure to heavy metal revealed Zn,Cu, and Fe stimulate growth whereas Pb and Hg caused significant inhibition. ($p<0.0001$, ANOVA). Response surface methodology (RSM) optimization enhanced degradation efficiency with GA3 strain degrading 75.9% glufosinate ammonium, the model shows great fit to the quadratic model (R2=0.9552) for GA3strain ($F=23.66$., $P<0.0001$). The results revealed that GA3 strain is capable of significant glufosinate ammonium degradation, with optimized conditions enhancing degradation efficiency. This study revealed great potential of indigenous soil microbes in bioremediation of herbicides-contaminated environments and provide a foundation for the development of eco-friendly strategies to mitigate glufosinate pollution in agricultural systems.

Keywords: Glufosinate Ammonium Degradation; *Bacillus* sp; bioremediation; OFAT; RSM; heavy metal; 16S rRNA analysis; phylogenetic analysis.

INTRODUCTION

The need for global food security is now more urgent than at any time in human history, and yet the need to protect environmental and human health are both necessary and challenging (Sharma *et al.,* 2019). Environmental pollution due to indiscriminate use of herbicides to control weeds has become a major problem in modern day agriculture. Thus, they accumulate in soil and water bodies and often obtain entry into the human food chain and cause diseases, reduce soil fertility and as well affect ecosystem balance. The use of herbicides as pre and post-emergence herbicides though harmful in several aspects, cannot be avoided Qamruzzamn *et al.,* (2017).

However, Glufosinate-ammonium, (GA) also, a non-selective herbicide for total vegetation control and as a desiccant to aid in crop harvesting, its herbicidal action is related to the inhibition of glutamine synthetase activity, an enzyme that plays an important role in ammonia detoxification, amino acid metabolism, protein and nucleotide biosynthesis in plants. The herbicide glufosinate ammonium is derived from phosphinothricin, a toxin isolated from the fungi *Streptomyces viridochromogenes* and *Streptomyces hygroscopicus* (Dayan and Duke, 2014). Plants susceptible to ammonium glufosinate exhibit glutamine deficiency, poisoning by ammonia accumulation, glutamate and glyoxalate accumulation, rupture of chloroplast structure, decreased electron transport, and inhibition of photosynthesis (Carbonari *et al.,* 2016

Glufosinate disrupts both photorespiration and the light reactions of photosynthesis, leading to photo reduction of molecular oxygen, which generates reactive oxygen species (*Takano and Dayan, 2020*). Glufosinate

ammonium (GA) degrades into harmful metabolites in different environmental conditions. In air, it forms methylphosphinic acid (MPA), which is persistent and can bioaccumulate (Ge *et al.,* 2023). In water, it breaks down into 3-methylphosphinicopropionic acid (MPP) and glutamic acid, both toxic to aquatic life (Zhou *et al.,* 2021). Under light, GA produces formic acid, ammonia, and nitrogenous compounds, which can contribute to eutrophication and alter soil and water chemistry (Singh *et al.,* 2022). Agricultural use of Glufosinate Ammonium is not allowed in the EU as its approval expired 31st July, 2018 (Bohn *et al.,* 2020). Microorganisms help in the breakdown, decomposition, and increase productivity, this herbicide is toxic to many of them and increases susceptibility to plant diseases. Long-term use of herbicide gives rise to herbicide-resistant weeds and therefore the production units are insisted to produce more strong herbicides, this results in more toxicity and depletes the sustainability of soil, fields, and seeds. Hence, these herbicides residues decontamination becomes important, the breakdown of glufosunate ammonium can be achieved using abiotic and biotic means such as photolysis, absorption, thermolysis and biodegradation by catabolic enzymes. However, an environmentally friendly strategy like biodegradation could be a promising approach to overcome the environmental and health risks caused due to glufosinate ammonium. Bioremediation exploits the ability of microorganisms to degrade and detoxify organic contaminants (muhammed *et al*., 2022). The agricultural application of glufosunate ammonium is associated with a significant risk to the soil, aquatic system and water resources. Thus the treatment glufosunate ammonium, after the fulfillment of its herbicidal applications, is essential to eliminate or at least minimize its negative impact. Muhammed *et al.,* (2023) opined that Microorganisms such as bacteria and fungi are the key agents of bioremediation, factors influencing the degradation, such as the content and concentration of the pollutants, the physicochemical environmental conditions and the composition of the microbial consortia, decide the rate of the overall microbial degradation process.

MATERIALS AND METHOD

Reagents and materials

petri dish, autoclave, conical flask, wire loop, hand lens, mineral salt medium, glufosinate ammonium, weighing balance, distilled water, incubator, shaker, soil sample, vijou bottles, fridge, Bacterial smear on glass slide (heat-fixed), Crystal violet (primary stain), Iodine solution (mordant), 95% ethanol or acetone-alcohol (decolorizer), Safranin (counterstain), Distilled water, Simmon's citrate agar slant (contains citrate, ammonium salts, and bromothymol blue as indicator), MR-VP broth, Tryptone broth. Glufosinate ammonium mineral salt medium (GA-MSM) used in these research study contains the following compositions as described by (Ibrahim *et al.,* 2022, Lukman *et al.,* 2024) in g/L: $Na_2HPO_4.2H_2O$ 6.0, KH_2PO_4 3.0, NH_4Cl 1.0, $MgSO_4.7H_2O$ 0.2, $CaCl_2.2H_2O$ 0.1, and pH 7.

MSM agar (NA)

Nutrient agar was prepared by adding 7 g agar agar to glufosinate ammonium mineral salt media dissolved in 1 L distilled water and then autoclaved at 121ºC for 15 minutes at 15 psi.

Sample Collection

The soil sample were collected from three different farm land in Mubi North, a region prominent for agricultural activities that utilize glufosunate ammonium-based herbicides. The sample were collected using a sterile spatula to a depth of 6 to 8 cm below the soil surface; it was placed in a sterile plastic container and store in a cooler before transferring to the laboratory for analysis (Muhammad *et al.,* 2024).

Enrichment and Isolation of Degrading Bacterial Strains

GA degrading bacteria was isolated by measuring 10 g soil and the soil were added into 250 mL Erlenmeyer flasks containing 100 mL sterile MSM ($Na_2HPO_4.2H_2O$ 6.0, KH_2PO_4 3.0, NH_4Cl 1.0, $MgSO_4.7H_2O$ 0.2, $CaCl_2.2H_2O$ 0.1, and pH 7.) in triplicate supplemented with 10mg/L of GA, and incubate in a rotary shaker at 37°C and 100 rpm (Lukman *et al.,* 2024,). Two weeks following the incubation, 5 mL suspension from each replicate were transferred to the flasks containing fresh MSM supplemented with 10mg/L of GA. Similarly, four consecutive transfers were carried out by sub-culturing as described earlier. GA degrading bacteria were isolated from the last enrichment culture. Each colony was subjected to degradation rate, biochemical tests, Gram's staining, and molecular identification. (Fatima *et al.,* 2022, Lukman *et al*., 2024, Suleiman *et al.,* 2024).

Morphological and molecular identification of bacteria isolate

Microscopic Identification and Gram Staining

Gram staining was carried out according to the protocol of (Ekram *et al.,* (2020). Bacterial smears were prepared on clean, microscopic glass slides. The smears were then stained to differentiate between gram-positive and gram-negative bacteria. **Step by step process: s**mall drop of water on a clean glass slide was placed and sterile loop was use to transfer a small amount of the bacterial culture onto the drop. A thin smear was spread and made was allow to air dry completely, the slide was heat fix by quickly passing it through a flame 2–3 times. **Primary Stain:** the smear was flooded with crystal violet, it was allowed to stay for 1 minute, and it was gently rinsed with distilled water. **Mordant:** the smear was flooded

with iodine solution, it was allowed to stay for 1 minute and it was gently rinsed with distilled water. **Decolorization:** decolorizer (ethanol) was apply (drop by drop) for about 10–20 seconds until runoff is almost clear. Immediately it was rinsed with distilled water to stop the decolorization. **Counterstain:** Safranin, the smear was flooded with safranin, it was allowed to stay for 1 minute and rinsed with distilled water, it was blot dry gently with absorbent paper. After the slides were completely dried a drop of immersion oil was placed on the slide and view under the x100 objective lens of a light microscope which can distinguish between gram positive and gram negative bacteria as well as provide information about the cell shape, presence of flagella or spore (Lukman *et al.,* 2024, Suleiman *et al.,* 2024).

Biochemical Identification of the Isolate (principle of test and procedure)

Biochemical tests were determined Based on protocol of Bergey's manual of determinative Bacteriological studies, the test includes catalase, oxidase, indole, citrate and sugar fermentation were used to classify and identify the bacterial species (Lukman *et al.,* 2024, Suleiman *et al.,* 2024).

Citrate utilization test: Using an inoculating needle, a colony of the test organism was lightly touched, the slant surface of Simmon's citrate agar was streak and Incubate at 35–37°C for 24–48 hours, the color change was observed.

$$\text{Citrate (outside)} \xrightarrow{\text{Citrate permease}} \text{Citrate (inside the cell)} \xrightarrow{\text{Citrase}} \text{Oxaloacetic acid + acetic acid}$$

$$\text{Oxaloacetic acid} \longrightarrow \text{Pyruvate} + CO_2$$

Methyl Red (MR) test: The MR-VP broth was incubated with the test organism at 35-37^{0}C for 48 hours, 5 drops of methyl red indicator were added to the culture and the tube was shake gently. The color change was observed.

$$\text{Glucose} \xrightarrow{\text{Glycolysis}} \text{Pyruvate} \xrightarrow{\text{Mixed acid fermentation}} \text{Acids (or acidic end products)}$$

Voges-Proskauer (VP) Test: The MR-VP broth was incubated with the test organism at 35-37^{0}C for 48hours, 0.6 mL (about 15 drops) of VP reagent A was added (alpha-naphthol) and 0.2 mL (about 5 drops) of VP reagent B (40% KOH) was added to the culture and the tube was shake gently for about 1 minute. The color change was observed.

$$\text{Glucose} \xrightarrow{\text{Glycolysis}} \text{Pyruvate} \xrightarrow{\text{Butanediol pathway}} \text{Acetoin (intermediate product)}$$

$$\text{Acetoin} \xrightarrow{O_2\ KOH} \text{Diacetyl} + H_2O$$

$$\text{Diacetyl + guanidine component of peptone} \xrightarrow{\alpha\text{ - napthol}} \text{Pink – red colour}$$

Catalase Test: Hydrogen peroxide were added to a bacterial culture. Produced bubbles indicate a positive result.

Indole Production Test: The bacterium was culture in a medium containing tryptophan. Kovac's reagent were added after incubation, and a red color indicate a positive result.

$$\text{Tryptophan + water} \xrightarrow{\text{Tryptophanase}} \text{Indole + pyruvic acid + ammonia}$$

Molecular Identification of Bacteria Isolate

DNA extraction; DNA extraction and amplification of 16S rRNA region of bacteria isolate were conducted based on the method describe by (Birniwa *et al*.,2012, Lukman *et al.,* 2024). The genomic extraction was conducted using the traditional phenol-chloroform method. Initially, the samples suspension was made in sterile distilled water and the resulting material was mixed with 200 µL of an extraction buffer and 20 µL of proteinase K, then left to incubate at 55°C overnight to

facilitate cell lysis. Subsequently, the chloroform-isopropanol extraction process was initiated by adding 200 µL of chloroform: isoamyl alcohol (24:1) solution to the sample. The mixture was centrifuged at 12,000 g for 5 minutes, leading to the separation of aqueous and organic phases. The upper aqueous phase, which contained DNA, was meticulously collected using a 200 µL pipette and transferred to a new 1.5 µL micro-centrifuge tube. The DNA was then precipitated by adding 200 µL of cold 70% ethanol, followed by centrifugation at 12,000 g for 5 minutes, resulting in the formation of a DNA pellet. This pellet was washed with ethanol to eliminate impurities. After air-drying to remove any remaining ethanol, the DNA pellet was dissolved in distilled water. Finally, the DNA was stored at -20°C for preservation.

PCR amplification; Amplification of 16S RNA was done by PCR, as stated by Jagaba *et al*., 2022, Lukman *et al.,* 2024, Suleiman *et al.,* 2024. The PCR result was run on agarose gel 1.5% stained with ethidium bromide. PCR amplification was carried out using Taq Mix (2x) (Master Mix) and universal primers 27F (forward) and 1492R (reverse), targeting the 16S rRNA gene. The PCR reactions were set up using the following parameters: Initial denaturation at 95°C for 5 minutes, denaturation at 95°C for 30 seconds, annealing at a 59°C for 30 seconds., extension at 72°C for 30 seconds, steps 2-4 were repeated for 30 cycles, final extension at 72°C for 7 minutes, and final hold at 4 °C.

Gel Electrophoresis: To verify the success of PCR amplification and check for the presence of 16S rRNA amplicons, a 1.5% agarose gel was prepared as follows; A 1.5% agarose gel powder was weighted and dissolved in 100ml of 1X TAE (Tris-Acetate-EDTA) buffer, followed by heating in a Microwave oven for 1 minute to form a homogenous solution. The homogenous molten agarose gel was then stained with 2 uL ethidium bromide to aid visualization under UV light. The gel was casted in a gel casting tray and allowed to solidify. Comb slots were inserted at one end of the gel for well formation. After gel solidification, the comb was gently removed, creating wells for sample loading. 4 uL of the PCR products were carefully loaded into the wells. Electrophoresis was conducted at 100V until the DNA bands migrated sufficiently (10 minutes). The electrophoresis gel was viewed under UV-Transluminator to check for bands (Lukman *et al.,* 2024, Suleiman *et al.,* 2024).

16S RNA Sequencing: The purified DNA was sent to Inqaba Biotechnology company, Nigeria for sequencing. Same primers that were earlier synthesized by Inqaba Biotech company were used for the sequencing. The 16S rRNA sequence obtained from the sequencing was compared to known 16S rRNA gene sequences in the non-redundant database using the BLAST 2. (Lukman *et al.,* 2024, Suleiman *et al.,* 2024, Yusuf *et al.,* 2024).

Phylogenetic analysis: Eleven closely interrelated 16S rRNA gene sequences recovered from Gen-Bank were compared with isolates and aligned using ClustalW Jagaba *et al*., (2022). The phylogenetic tree was constructed by using the neighbour-joining method of 16S rRNA gene sequences were compared in the Basic Local Alignment Search Tool (BLAST). The aligned closely related species were used to construct a Maximum likelihood tree using the MEGA X software with bootstrap method (Harun *et al.,*2023, Alhassan *et al.,* 2020).

Optimization of growth and degradation using one-factor-at-a-time (OFAT)

Parameters to be optimized include temperature, incubation time, substrate concentration, pH, carbon source, nitrogen source and inoculum size. The factors (pH, concentration, temperature, inoculum size, carbon source, nitrogen source and incubation time) that affect the growth of the bacteria isolate during herbicides degradation were characterized based on one factor at a time (OFAT). Replicate experiments were carried out (Manogaran *et al.,* 2018, Suleiman *et al.,* 2024,)

Effect of Incubation Time

The effect of time on bacteria growth were observed at 0 h, 24 h, 48 h, 54 h, 72 h and 96 h. 100mL of MSM were dispensed into the conical flask and inoculated with 100 µL bacteria suspension. Optical density (OD) at 600-620nm were measured at 0 h, 24 h, 48 h, 54 h, 72 h and 96 h to determine bacteria growth.

Effect of Glufosunate Ammonium Concentration (Substrate Concentration)

Glufosunate ammonium was utilized as the carbon source, the concentration of the pollutant was increased, varying the range from 5 – 200mg/L (5mg/L, 10mg/L, 20mg/L, 50mg/L, 100mg/L and 200mg/L).

Effect of Inoculum Size

The inoculum size ranging from 0.5% to 10% (0.5%, 1%, 3%, 5%, and 10%) were used to determine the optimum inoculum needed for bacteria growth.

Effect of Initial pH

The pH of the medium was set from 5 – 8.5 (5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5) to determine the optimum pH for the growth of the bacterium.1 M NaOH and 50 % HCl were used to adjust to various pH.

Effect of Temperature

The temperature was ranged from 25 – 45 ºC (25^{0}C, 30 ºC, 35 ºC, 40 ºC and 45 ºC) to determine the best

temperature that supports bacteria growth of bispyribac sodium and glufosunate ammonium degradation.

Effect of Carbon Sources

Carbon source is one of the most vital requirement for bacteria to grow, in order to check if the presence of additional carbon source to the glufosinate ammonium (sole carbon source) in glufosinate ammonium MS media might stimulate the bacterial growth/degradation or inhibit it. The carbon sources that were tested includes Fructose, Glucose, Maltose, Mannose, Sucrose, Lactose and Citric acid. The Negative control used for the carbon source was comprising of minimal salt media with the absence of Carbon source.

Effect of Sucrose Concentration

Sucrose being found to be the best Carbon source was tested at concentrations ranging from 0.5, 0.75, 1, 1.5, 2 to 3.0 g/L. This plot of bacterial growth against sucrose concentration was drawn to determine the best sucrose concentration.

Effect of Nitrogen Source

The nitrogen sources trialled were ammonium sulphate, aspartic acid, urea, phenylalanine, sodium nitrate, Uric acid, glutamic acid and ammonium chloride, a plot of bacterial growth against nitrogen sources was plotted. The Negative control used for the Nitrogen sources was comprising of minimal salt media with the absence of Nitrogen source.

Effect of Glutamic Acid Concentration

Glutamic acid as the best nitrogen source was tested at concentrations ranging from 0.5, 0.75, 1, 1.5, 2 to 3.0 g/L. The plot of bacterial growth against glutamic acid concentrations showed the optimum glutamic acid concentration

Statistical Optimization using Response Surface Methodology (RSM)

Two stages of statistical method of optimization of glufosunate ammonium-degrading bacteria isolated was done using Design-Expert software (v13). All experiments were carried out in triplicates with the average taken as the response. Percentage of glufosunate ammonium degradation were used as response (Yakasai *et al.,* 2020, Babandi, *et al.,* 2021). The same parameters investigated using OFAT were studied using RSM. The final RSM predicted response was further validated experimentally.

Plackett-Burman Design

Plackett-Burman design was employed as a screening design in order to estimate main effects of the factors on the response variable i.e. bacterial growth. It consists of 12-run design when screening more than four factors. It was developed by entering the factors i.e. temperature, pH, inoculum size, initial glufosinate ammonium concentration, glutamic acid concentration and sucrose concentration and setting their low (-1) and high (+1) levels. The Design Expert (v13) software generated 12 experimental runs in a randomized order. The 12 experimental runs were carried out and values inputed into the software after which the result was analysed to determine significant factors which were further optimized to find optimal conditions with central composite design (Ibrahim *et al.,* 2020, Suleiman *et al.,* 2024).

Central Composite Design

Central composite design was employed to determine the interaction effects of factors on each other against the response variable (bacterial growth). After the significant factors were identified in PBD, central composite design was set up using Design Expert software (v13) which uses the significant factors identified previously with five levels of the factors, which are; low (-1), high (+1), center (0), axial low and high (-±α). This provided full CCD matrix and the experiments were carried out after which the appropriate model was fit considering p-value as <0.05 to be significant (Yakasai *et al.,* 2020, Ibrahim *et al.,* 2020, Babandi *et al.,* 2021).

Effect of heavy metals

The effect of different heavy metals on potential isolate(s) was studied in order to ascertain their stimulatory or inhibitory effect. Heavy metals salts (MERCK, Germany) viz. Fe, Zn, Cd, Cr, Mn, Cu and Pb with the 1 ppm concentration was added into the glufosinate ammonium liquid media. The media was then incubated in an optimized condition based on OFAT and RSM. The amount of bacterial growth was measured. All the experiments were conducted in triplicates and the results obtained for glufosinate ammonium degradation was compared to observe whether or not these metals caused a decrease in degradation (Alhassan *et al.,* 2020, Fatima *et al.,* 2022, Usman *et al.,* 2023).

Measurement of Degradation Rate

Seed Culture

Bacterial strains were grown in LB medium. The overnight grown bacterial cultures was centrifuged for 10 min at 4600×g. Seed cultures was prepared by cell pellets was washed with normal saline (0.9% NaCl) and suspended in the same solution to set an OD of 0.8 at 600 nm. Colony forming units (CFU mL-1) was measured by dilution plate count method. For GA degradation studies, 2 % of these seed cultures were used as inoculum.

Glufosinate ammonium (GA) degradation rate of pure strains

GA degradation studies, experiments were carried out in 250 mL Erlenmeyer flasks containing 100 mL MSM ($Na_2HPO_4.2H_2O$ 6.0, KH_2PO_4 3.0, NH_4Cl 1.0, $MgSO_4.7H_2O$ 0.2, $CaCl_2.2H_2O$ 0.1, and pH 7), supplemented with 10 mg/L GA. Seed cultures of strain

were inoculated and flasks were incubated at 37 °C in a rotary shaker at 100 rpm for 15 days. The experiment was performed in triplicate were un-inoculated flasks served as control. Samples were harvested and measured for residual GA concentration. LC-MS equipped with variable wavelength UV-detector, column oven, a ternary gradient pump, electric sample valve, and ODS2 C18 reversed phase column was used for sample analysis. The mobile phase was acetonitrile and deionised water (55:45, v/v) with a flow rate of 1 ml/min and the sample injection volume was 20 µl. GA was detected and quantified at 250nm with its retention time of 5.7 min (Lukman *et al.,* 2024).

RESULT AND DISCUSSION

Isolation of Glufosinate Ammonium-Degrading Bacteria

A total of 12 bacterial colonies were isolated from the GA soil samples of which 6 were able to grow on both glufosinate ammonium MS liquid and glufosinate ammonium MS solid media containing 10 mg/L glufosinate concentration, thereby indicating their ability to used glufosinate ammonium as a sole carbon source.

Screening of Best Glufosinate Ammonium-Degrading Bacteria

The result in figure 1 below shows plot of bacterial growth obtained from mineral salt medium containing glufosinate ammonium herbicides measured by UV-spectrophotometer against the GA isolate. Isolate GA3 shows the highest growth and hence the best glufosinate degrading bacteria when compared to the other isolate follow by isolate GA2. Isolate GA3 showed the highest bacterial growth after 48 hours. All the remaining isolate showed lower growth with isolate GA1 showing the least growth of them all.

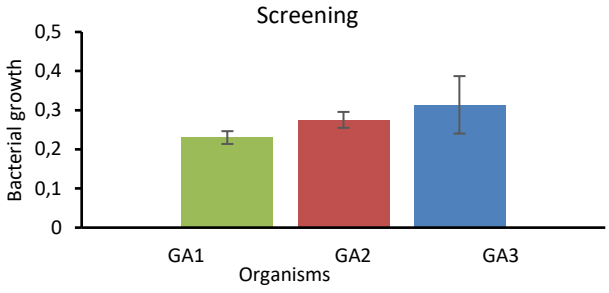

Figure 1. Plot of bacterial growth.

Identification of glufosinate ammonium degrading bacteria

Morphological and Biochemical Identification

Gram staining

Figure 2 below shows colony morphology, cell organization, gram staining reaction, microscopic observation, isolate GA3 retained crystal violet stain that visually confirms the isolate was gram-positive rod shaped bacilli due to the appearance of purple coloration of the rod-shaped after viewed under the ×100 oil immersion objective lens.

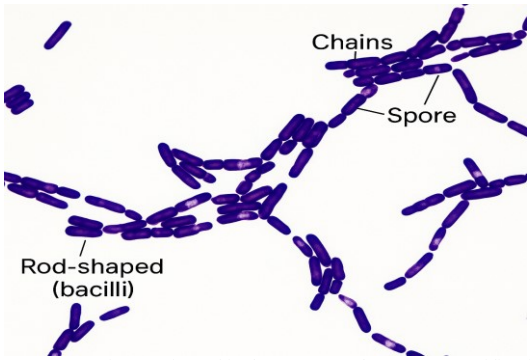

Figure 2. Gram stain reaction of isolate GA3 under x100 magnification.

Biochemical identification

The biochemical tests employed during the course of the research include: voges proskaeur, indole, citrate utilization, methyl red, catalase and oxidase test.

Table 1 Morphological and biochemical characteristics of isolate GA3, which indicate it belong to the genera *Bacillus.*

ID	Biochemical analysis	Characteristics
Isolate GA3	Indole	+
	Methyl red	+
	Voges proskauer	_
	Citrate	+
	Oxidase	_
	Catalase	+

Molecular 16S rRNA Identification

Genomic DNA Extraction

Majority of bacteria have a genome that consists of a single DNA molecule (i.e., one chromosome) with several million base pairs in size. therefore, bacteria may have one or more smaller circular DNA molecules, called plasmids. The genomic DNA of the isolate were successfully extracted and analysed on 1% (w/v) agarose gel (Figure 3).

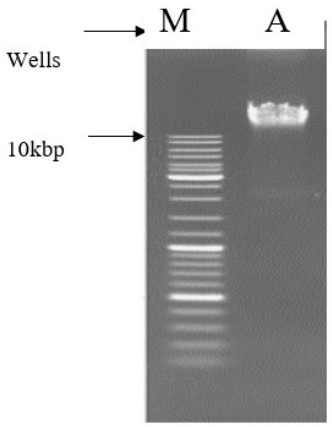

Figure 3. Genomic DNA of *Bacillus sp* on 1% (w/v) Agarose gel.
NB: M = Marker, A = *Bacillus sp.*

Amplification of 16S rRNA gene by Polymerase chain reaction

In the PCR process, the genomic DNA that was obtained from the genomic DNA extraction process was used as a template in the amplification of the 16S rRNA gene. Both the universal primer used in the amplification of the gene was synthesized by from Inqaba Biotec. The PCR product was approximately 1.5 kb as shown in Figure 4 observed using 1.0 % agarose gel electrophoresis and sent for DNA sequencing.

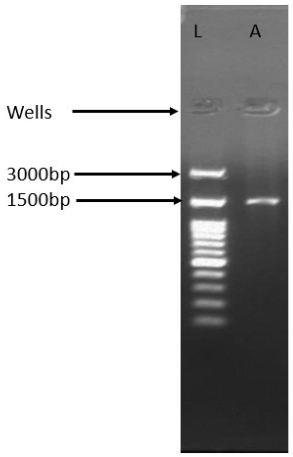

Figure 4. Agarose gel electrophoresis of PCR product of 16S rRNA gene of isolate L= Ladder, A = Isolate GA3.

Phylogenetic Tree construction

The phylogenetic tree constructed in MEGA 11 using the neighbor joining tree method, and it depicted the bacterial isolate, GA3 cluster with *Bacillus* spp as shown in Figure 5 below. This finding supported that the isolate is important bacteria found in the environment that have been isolated and characterized to have potential to degrade glufosinate ammonium (Fareed *et al.,* 2017). Isolate GA3= *Bacillus* spp

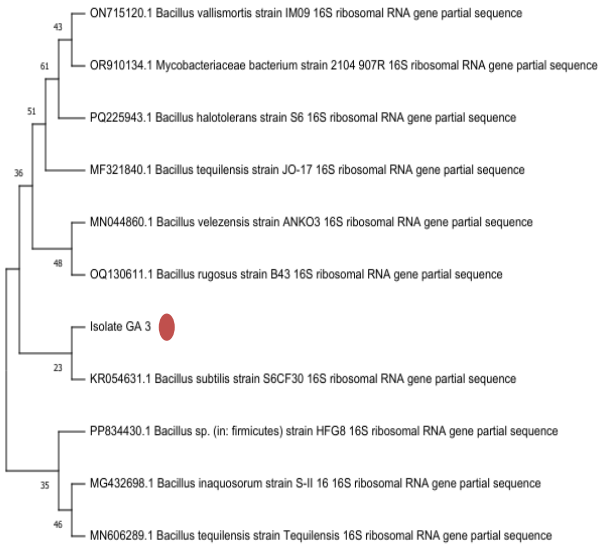

Figure 5. Phylogram (Neighbour-joining method) indicating the 16s rRNA genetic relationship between 11 other related reference bacteria from the NCBI database and isolate GA3.

Optimization of bacterial growth One Factor at a Time (OFAT)

Effect of incubation time

The time frame for the bacterial growth used was 0, 24, 48, 72 and 96 hours. Plot of bacterial growth against incubation time provided a growth curve which signifies that the isolate had their highest growth using OD_{600} nm measurement after 48 hours of incubation for isolate GA3 (*Bacillus* sp.) (Figure 6).

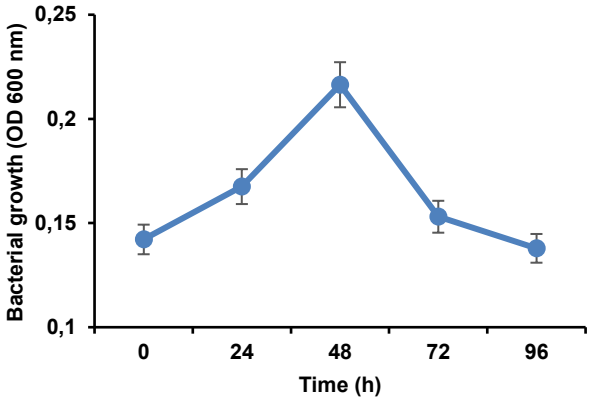

Figure 6. The effect of incubation time on bacterial growth of *Bacillus* sp. at 35°C, 10 mg/L initial glufosinate ammonium concentration, 5% inoculum size and pH 7. Error bars represent standard deviation, n = 3.

Effect of substrate concentration

The effects of initial concentrations (5, 10, 20, 50, 100 and 200 mg/L) on bacterial growth rate were studied. Optimum growth after 48 h incubation time was obtained at an initial concentration of 10mg/L shown on Figure 7 with growth reduced rapidly at concentration above 20mg/L.

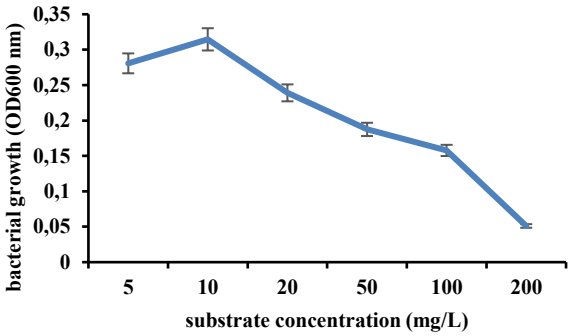

Figure 7. The effect of substrate concentration on bacterial growth of *Bacillus* sp at 35°C, 5% inoculum size, 10mg/L substrate concentration and after 48 h incubation. Error bars represent standard deviation, n = 3.

Effect of pH

pH an essential parameter that affects the growth and metabolism of all bacteria. The effects of pH on bacterial growth was studied at different pH values ranging from pH of 5, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 to 8.5, and an optimum pH of 7 was identified as seen in (Figure 8).

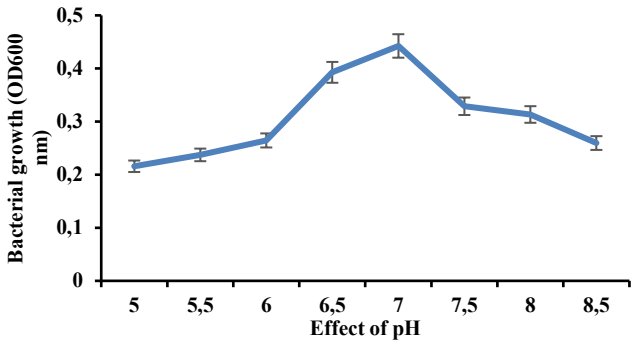

Figure 8. The effect of pH on bacterial growth of *Bacillus* spp at 35°C, 5% inoculum size and 10mg/L initial glufosinate ammonium concentration after 48 h incubation. Error bars represent standard deviation, n = 3.

Effect of temperature

Temperature is the most critical physical factor affecting bacterial growth and is a key variable in optimizing microbial growth and biodegradation. The optimum temperature for bacterial growth (OD_{600}) measured in this study was 35°C, above which temperature the growth declined (Figure 9)

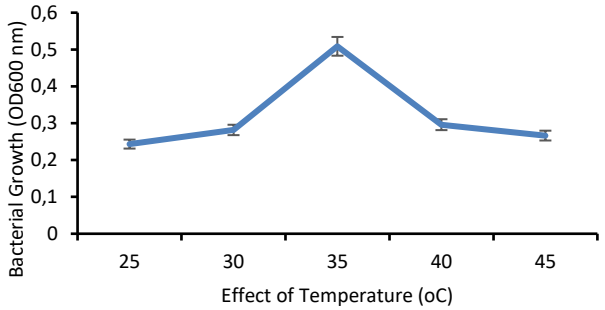

Figure 9. The effect of temperature on bacterial growth of *Bacillus sp* using 10 mg/L initial glufosinate ammonium concentration, 5% inoculum size and pH 7 after 48 h incubation. Error bars represent standard deviation, n = 3.

Effect of inoculum size

The effect of inoculum size was studied over a range of 0.5, 1.0, 2.0, 3.0, 5.0 to 10% (v/v). Bacterial growth was optimum with inoculum size of 5% (v/v), after which there was a rapid decline as shown in Figure 10.

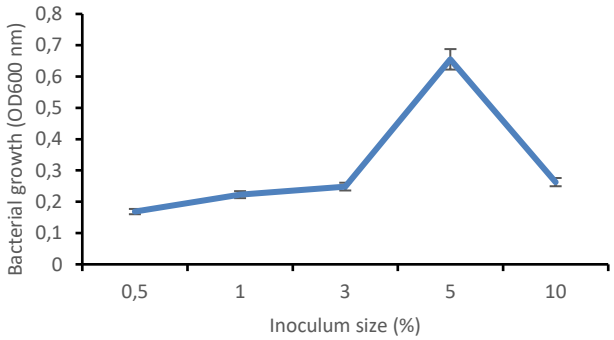

Figure 10. The effect of inoculum size on bacterial growth of *Bacillus* spp at 35°C, 5% inoculum size and 10mg/L initial glufosinate ammonium concentration after 48 h incubation.at 35°C, 5% inoculum size and 10mg/L initial glufosinate ammonium concentration after 48 h incubation. Error bars represent standard deviation, n = 3.

Effect of nitrogen source

Nitrogen source is an important elements influencing bacterial growth. Different nitrogen sources may have inhibitory or stimulatory effects on bacterial growth. Out of the eight organic and inorganic nitrogen sources tested here on GA exhibited maximum growth when glutamic acid was used, which signifies that the organism prefers glutamic acid as a nitrogen source as seen on Figure 11.

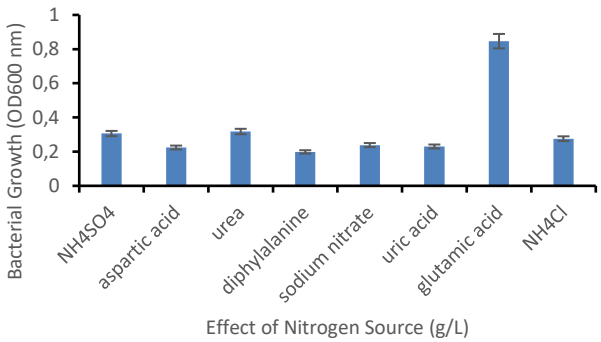

Figure 11. The effect of nitrogen source on bacterial growth of *Bacillus* sp at 35°C, 5% inoculum size and 10mg/L initial glufosinate ammonium concentration after 48 h incubation. Error bars represent standard deviation, n = 3.

Effect of glutamic acid concentration

The effects of different glutamic acid concentrations on bacterial growth was investigated at levels of 0.5, 0.75, 1, 1.5, 2, and 3 g/L, indicated that 1.0 g/L was the optimum glutamic acid concentration as shown in Figure 4.12. At a concentration lower than the optimum, the rate of growth of the bacteria decreased, presumably due to insufficient aspartic aci being available to sustain the replication rate of the bacterium likewise at concentration higher than 1.5 g/L there was a sharp decline in the bacterial growth of both the isolate GA3 This indicate that both the organisms utilize similar pathways for their metabolism. Figure 12 below show the effect of glutamic acid concentration on Bacterial growth.

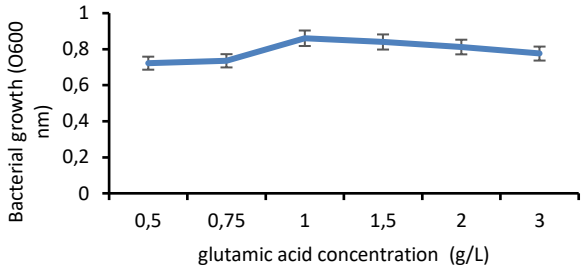

Figure 12. The effect of glutamic acid concentration on bacterial growth of *Bacillus* sp at 35°C, 5% inoculum size and 10mg/L initial glufosinate ammonium concentration after 48 h incubation. Error bars represent standard deviation, n = 3.

Effect of carbon source

Carbon source is an important elements influencing bacterial growth. Different carbon sources in addition to the glufosinate ammonium being degraded may have inhibitory or stimulatory effects on bacterial growth. All

the carbon sources that were tested here indicate to have a stimulatory effect on the bacterial growth. Sucrose was found to be the most preferred carbon source that stimulated the bacterial growth of the isolate which signifies that organism prefers sucrose as a carbon source as shown on Figure 13.

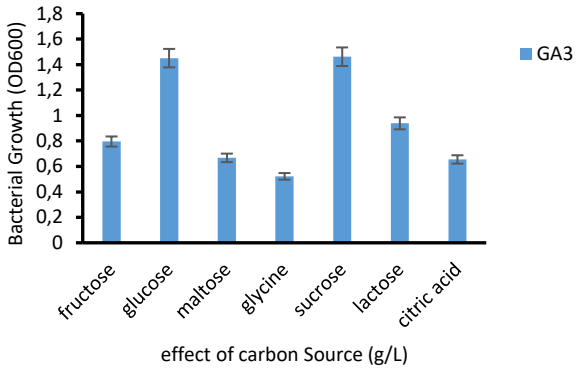

Figure 13. The effect of carbon source on bacterial growth of *Bacillus* sp at 35°C, 5% inoculum size and 10mg/L initial glufosinate ammonium concentration after 48 h incubation. Error bars represent standard deviation, n = 3.

Effect of sucrose concentration

The effect of sucrose concentration on bacterial growth was studied at sucrose concentrations of 0.5, 0.75, 1, 1.5, 2, and 3 g/L. 1g/L concentration of sucrose was found to be the most optimum for isolate GA3 as shown on Figure 14.

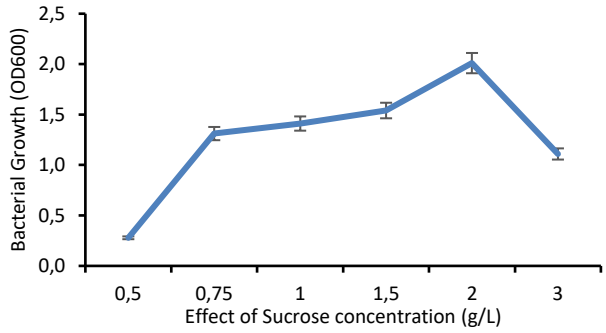

Figure 14. The effect of sucrose concentration on bacterial growth of *Bacillus* sp at 35°C, 5% inoculum size and 10mg/L initial glufosinate ammonium concentration after 48 h incubation. Error bars represent standard deviation, n = 3.

Response Surface Methodology

Plackett-Burman Design

PBD was used to screened and select the significant parameters affecting glufosinate ammonium degradation. The approach can be applied to a number of independent parameters in one experiment (Plackett and Burman, 1946). The ranges used for each of the independent parameters selected were chosen based on the ranges used in OFAT, and a total of 12 experiments were conducted in PBD using state parameters as shown in Table 2 and 3 below.

Table 2. Experimental design, and bacterial growth achieved, in 12 experimental runs of Placket–Burman Design applied to glufosinate ammonium degradation isolate GA3 (*Bacillus sp*).

Run	A	B	C	D	E	F	Bacterial growth (OD 600nm)
1	40	7.5	6	5	1.5	0.75	0.9806
2	30	7.5	4	5	2.5	1.5	0.538
3	40	6.5	4	12	1.5	1.5	0.9978
4	30	6.5	4	5	1.5	0.75	0.2164
5	40	7.5	4	12	1.5	1.5	0.9139
6	30	6.5	6	12	2.5	1.5	0.627
7	30	7.5	4	12	2.5	0.75	0.3109
8	40	6.5	6	12	2.5	0.75	1.17
9	30	6.5	6	5	1.5	1.5	0.4472
10	40	7.5	6	5	2.5	1.5	1.3301
11	30	7.5	6	12	1.5	0.75	0.1649
12	40	6.5	4	5	2.5	0.75	1.1488

A = Temperature (°C), B = pH, C = Inoculum size (% v/v), D = Initial glufosinate ammonium concentration (mg/L), E = Carbon source (g/L), F = Nitrogen source (g/L)

PBD is useful for fitting first order models (which identify linear effects) and can give an indication of the existence of second-order curvature effects when the design includes centre points (Ibrahim *et al.,* 2015). In PBD, a total of 12 different experiments were required with the six parameters, saving time, resources and energy, and the response is larger when compared with full factorial design. The model F value confirms that the models were significant as shown in Table 2. The model revealed that, of the parameters used for Isolate GA3 (Bacillus spp) pH, inoculum size, and substrate concentration were not significant parameters.

Table 3. Analysis of variance for strain GA3 bacterial growth for glufosinate ammonium degradation from PBD.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.78	10	0.1783	1254.33	0.0220	Significant
A-Inoculum size	0.0158	1	0.0158	111.12	0.0602	
B-pH	0.0072	1	0.0072	50.76	0.0888	
C-Substrate concentration	0.0080	1	0.0080	55.98	0.0846	
D-Carbon source	0.0426	1	0.0426	299.56	0.0367	
E-Nitrogen source	0.0276	1	0.0276	194.42	0.0456	
F-Temperature	0.8510	1	0.8510	5986.85	0.0082	
AB	0.0000	1	0.0000	0.1520	0.7634	
AD	0.0001	1	0.0001	0.8690	0.5223	
AE	0.0002	1	0.0002	1.67	0.4195	
BC	0.0010	1	0.0010	6.95	0.2308	
Residual	0.0001	1	0.0001			
Cor Total	1.78	11				
Std. Dev.	0.0119		R^2	0.9999		
Mean	0.7371		Adjusted R^2	0.9991		
C.V. %	1.62		Predicted R^2	NA(1)		
			Adeq Precision	102.3803		

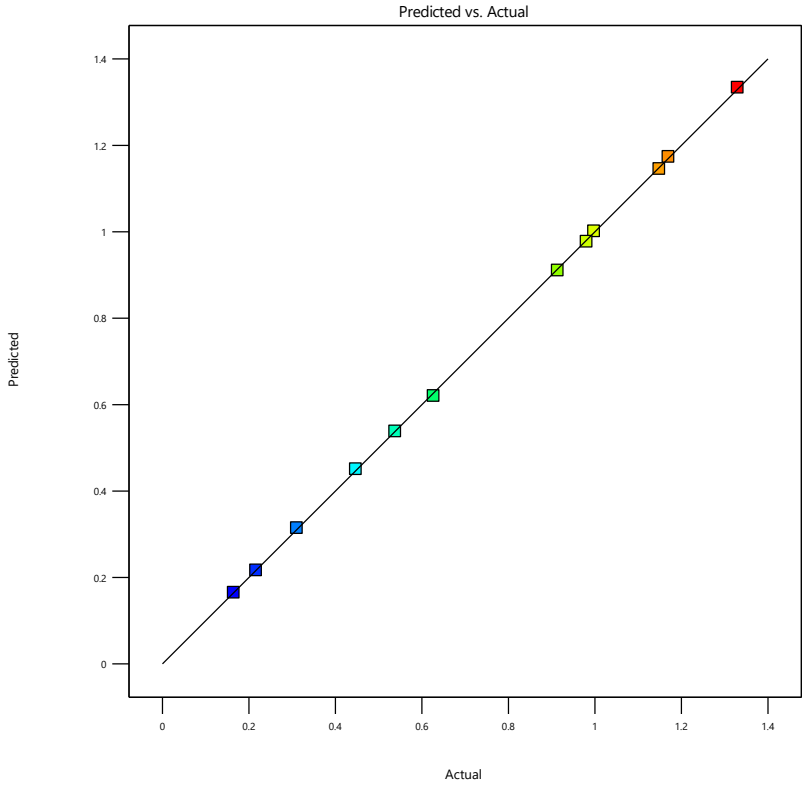

Figure 15. Similarity plot of predicted vs actual value of Bacterial growth for *isolate GA3* strain.

Central Composite Design CCD

Table 4. Input parameters for GA3.

Factor	Name	Units	Minimum	Maximum
A	Carbon source	g/L	0.3296	1.17
B	Nitrogen Source	g/L	0.4943	1.76
C	Temperature	ºC	26.59	43.41

Table 5. CCD experiment summary for GA3.

Run	Factor 1 A: Carbon source g/L	Factor 2 B: Nitrogen Source g/L	Factor 3 C: Temperature ºC	Response 1 Bacterial growth OD600	Predicted Bacterial growth
1	0.75	0.494328	35	1.076	1.08
2	0.5	1.5	40	0.9483	0.9526
3	1	1.5	30	0.5823	0.5849
4	1.17045	1.125	35	0.8857	0.8908
5	1	0.75	40	1.0441	1.05
6	0.75	1.125	26.591	0.6892	0.6889
7	0.75	1.125	35	0.8768	0.8962
8	0.75	1.125	35	0.8763	0.8962
9	0.75	1.125	43.409	0.7755	0.7785
10	0.5	0.75	30	1.0168	1.02
11	0.75	1.125	35	0.8738	0.8962
12	0.5	1.5	30	0.9988	0.9952
13	0.329552	1.125	35	0.9693	0.9670
14	1	0.75	30	0.9028	0.8965
15	0.75	1.125	35	0.8794	0.8962
16	0.75	1.125	35	1.002	0.8962
17	0.5	0.75	40	0.7306	0.7261
18	1	1.5	40	0.9964	0.9883
19	0.75	1.125	35	0.8696	0.8962
20	0.75	1.75567	35	1.0034	1.01

Table 6. CCD ANOVA for GA3.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.2919	9	0.0324	23.66	< 0.0001	significant
A	0.0070	1	0.0070	5.12	0.0472	
B	0.0062	1	0.0062	4.51	0.0596	
C	0.0097	1	0.0097	7.07	0.0239	
AB	0.0403	1	0.0403	29.41	0.0003	
AC	0.0995	1	0.0995	72.59	< 0.0001	
BC	0.0323	1	0.0323	23.58	0.0007	
A^2	0.0019	1	0.0019	1.40	0.2640	
B^2	0.0378	1	0.0378	27.58	0.0004	
C^2	0.0476	1	0.0476	34.71	0.0002	
Residual	0.0137	10	0.0014			
Lack of Fit	0.0002	5	0.0000	0.0184	0.9998	not significant
Pure Error	0.0135	5	0.0027			
Cor Total	0.3056	19				
Std. Dev.	0.0370		R^2	0.9552		
Mean	0.8999		Adjusted R^2	0.9148		
C.V. %	4.11		Predicted R^2	0.9296		
			Adeq Precision	18.7939		

NB: A-Carbon source, B-Nitrogen Source, C-Temperature

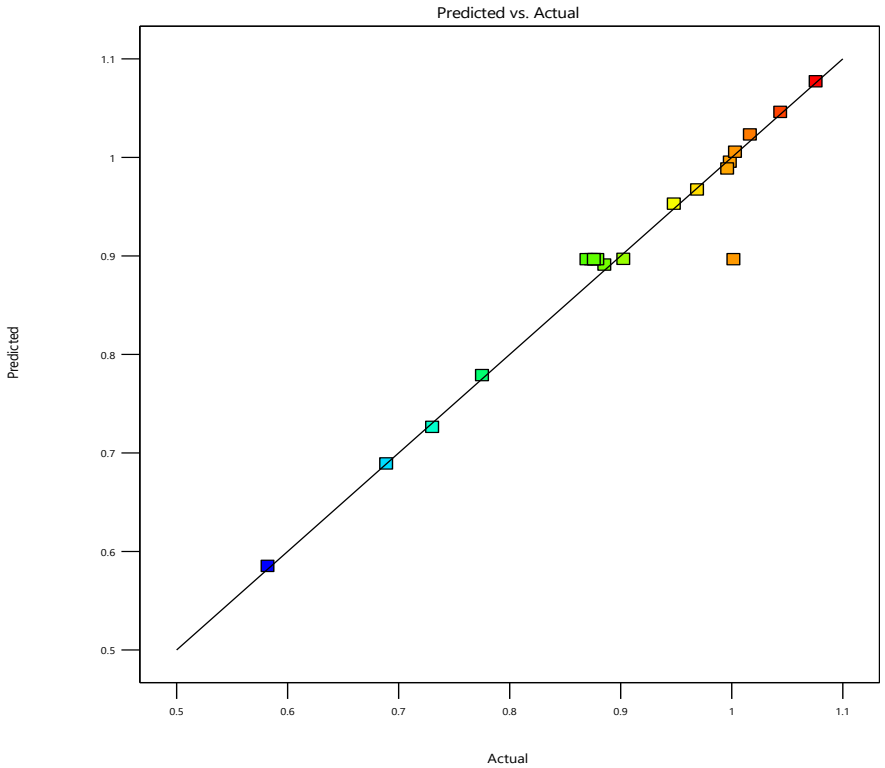

Figure 16. Similarity plot of predicted vs actual value of Bacterial growth for *isolate GA3* strain.

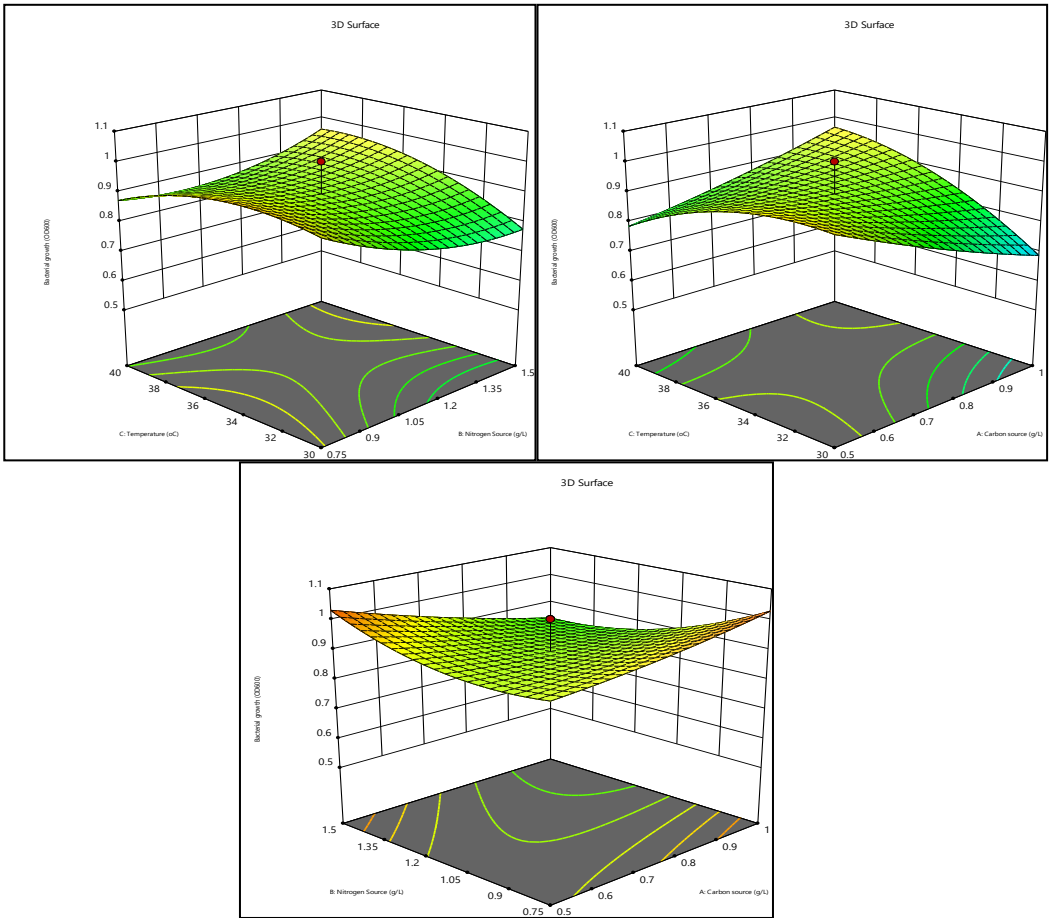

Figure 17. Relationship between Carbon source and nitrogen source on bacterial growth on strain GA3. Relationship between Carbon source and Temperature on bacterial growth on strain GA3, and relationship between Nitrogen source and Temperature on bacterial growth on strain GA3.

Effect of Heavy Metals

In Figure 18 below a graph shows the various effect of heavy metals on bacterial growth in relation with the control (growth media containing no heavy metals), as seen for *Bacillus spp* the metals Hg, Pb, Mn, Fe, Cu and Cd inhibit bacterial growth while Zn was found to stimulate bacterial growth.

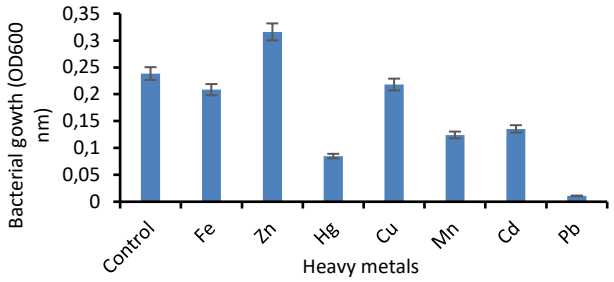

Figure 18. The effect of heavy metals on bacterial growth of *Bacillus* sp at 35°C, 5% inoculum size and 10mg/L initial glufosinate ammonium concentration after 48 h incubation. Error bars represent standard deviation, n = 3.

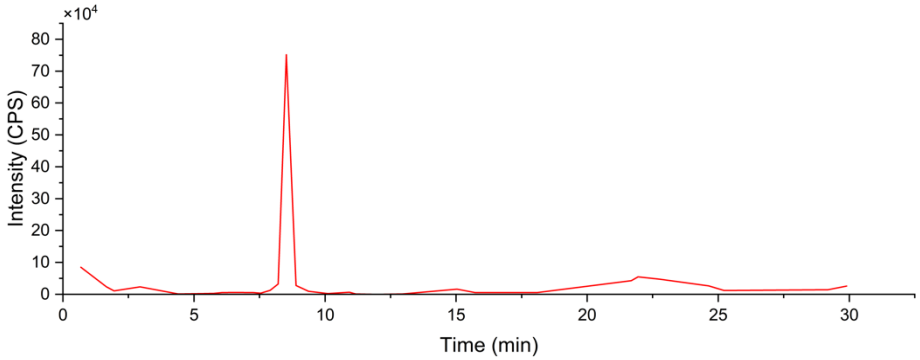

Figure 19. GA control LC-MS spectra (R_t=8.5min).

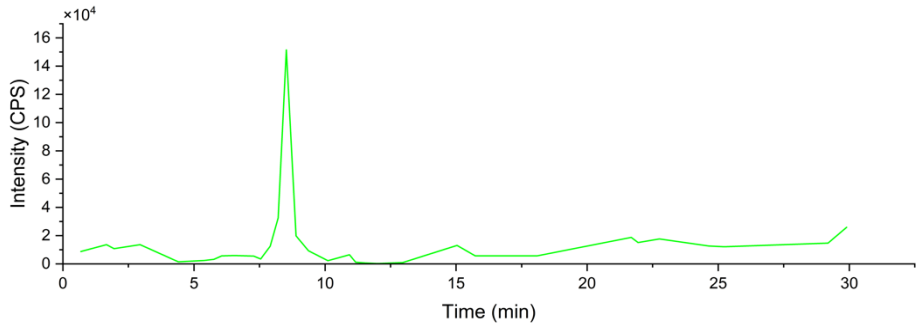

Figure 20. GA3 LC-MS spectra (R_t=8.5min).

$$\text{Percentage degradation} = \frac{a1-a2}{a1} * 100$$

Table 7. A1= peak area of control, A2 = peak area of sample.

S/N	Sample	Peak Area	Percentage Degradation
1	GA control	271736.5651	-
2	GA3	65398.72511	75.9%

Validation of Experiments

The validation of experiment for isolate GA3 was carried out by running a confirmatory experiment with the conditions: carbon source, 0.75g/L; Nitrogen Source, 1.125g/L, and Temperature, 35°C, while keeping the other factors constant after which the one sample T-test shows that there was no statistically significant difference (p = 0.001, t = 31.029) between the predicted mean (M = 0.8960) and observed mean (M = 0.9344) of isolate GA3.

DISCUSSION

Enriched glufosinate ammonium culture was found to support the growth of bacteria with GA- degrading ability. 3 isolate (GA1, GA2, and GA3) were found to be able to grow on both glufosinate ammonium liquid medium and GA solid medium proving its ability to

utilize glufosinate ammonium as sole carbon source which is similar to the work of Fareed *et al.,* (2017). The three isolate were subjected to a degradation studies and after a period of 96h, GA3 was found to be able to grow best in GA enriched media when compared to isolate GA1, GA2 as shown in Figure 1. One-way ANOVA identified that there is no significant overall difference between the different isolates ($F(2,6) = 2.592$, $p=0.154$). Since GA3 had the highest growth, therefore isolate GA3 was selected to be subsequently used for the study.

After the selection of best isolate, the isolate was then subjected to different tests. The isolate GA3 was identified as gram-positive by retaining stain (crystal violet) purple coloration as seen on Figures 2. Then based on biochemical characterization, isolate GA3 was provisionally suspected to be *Bacillus sp* on Table 1. The 16S rRNA amplification was conducted through PCR for the bacterial isolate that was biochemically identified, where extracted genomic DNA show that was amplified using 16S rRNA primers showed positive bands in 1% agarose gel (1500bp), extrapolated from the Ladder as shown in Figure 4. Finally, all the results were submitted to GenBank, and BLAST similarity and the phylogenetic analysis revealed that isolate GA3 was confirmed to be *Bacillus spp* as shown in Figures 5. This shares a similarity with the work of Hsaio *et al.,* (2007) where *Burkholderia sacchari and Serratia marcescens species* are isolates capable of degrading glufosinate ammonium.

Bacteria are known to adapt to a new environment, and after which they exponentially grow until a plateau is reached and decline phase sets in which follows the sigmoidal growth curve (Krishnamurthi *et al.,* 2021). In this study, the optimum bacterial growth was observed at 48 h of incubation for *Bacillus sp*. It was observed that the growth steadily increased until the optimum of 48 h was reached after which, a decrease in the bacterial growth, which could be attributed to the exhaustion of the glufosinate ammonium which served as carbon source and the accumulation of metabolic wastes, which could possibly have hindered the growth and degradation of the isolate. One-way ANOVA identified a significant overall difference between the time periods of *Bacillus* sp ($F(4, 10) = 3902.222$, $p < 0.0001$) Post-hoc test for *Bacillus sp* indicated that there was significant difference between the optimum time of 48 h ($M = 0.2164$, $SD = 0.0005$) to the remaining levels as shown in figure 6. The result obtained here align with the finding of Thongmee and Sukplang, (2024) who reported maximum growth at 48 h incubation for *Pseudomonas psychrophila* (MT163428.1) and *Pseudomonas putida* (MK878726.1).

Temperature normally supports the growth and activity of microorganisms, the growth and activity of microorganisms rapidly decline when temperature is high, because most microbes cannot survive at such high temperatures (Wani *et al.,* 2022). One-way ANOVA identified a significant overall difference between the temperature levels of *Bacillus sp* strain GA3 ($F(4,10) = 1872.753$, $p < 0.001$). Post-hoc test for *Bacillus sp* indicated that there was significant differences of bacterial growth mean values of the optimum temperature and the remaining temperature levels tested. In this study, the *Bacillus sp* obtained showed optimal growth and highest degradation at 35 °C. Temperatures of higher than 40 °C did not support the growth of the isolate which was characterized by a sharp decline in the bacterial growth as shown in figure 9. These findings align with the findings of Jahan *et al*. (2025) who reported the optimal growth at 37ºC was attained at mesophilic temperature. Mesophilic bacteria tend to have more benefits over thermophilic bacteria from an environmental standpoint. Mesophilic bacteria are also more resilient and adaptive to changing environmental circumstances than other bacteria.

pH on bacterial growth and metabolism efficient for fermentation, as pH influences the transfer of various nutrients in and out of the microorganism's cell membrane (Naz *et al.,* 2022). The bacterial growth of *Bacillus sp* was checked over a pH range between 5.5 and 8.5. Maximum bacterial growth was observed at the pH of 7.0 for the isolate. pH above which the growth diminished sharply as seen on Figure 8. One-way ANOVA identified a significant overall difference between the pH levels of *Bacillus sp* ($F(7.16) = 709.243$, $p < 0.001$). Post-hoc test for *strain* GA3 indicated that there were significant differences between pH 7 ($M = 0.4424$, $SD = 0.0135$) and the remaining pH levels tested.

Optimum inoculum size is essential for bacterial growth and metabolism. A higher inoculum (concentration of bacterial cells) during fermentation is beneficial to a certain extent for faster degradation after a certain level the inoculum is theorized to become too dense that competition between bacterial strains is intense and hence leads to slower degradation capability (Liu *et al.,* 2023). This is shown on Figure 10. A smaller inoculum size indicates that low bacterial cell density might take a longer period to replicate and reach optimum level for extensive biodegradation (Liu *et al.,* 2023). One-way ANOVA identified a significant overall difference between the inoculum size levels of *Bacillus* sp ($F(4.10) = 5666.595$, $p < 0.001$). Post-hoc test for *strain* GA3 indicated that were significant differences between optimum inoculum size 5% ($M = 0.6550$, $SD = 0.0072$) and the remaining inoculum size levels tested. In this study of the different inoculum concentration (0.5%, 1%, 3%, 5% and 10%) used the highest bacterial growth was observed at 5% inoculum size which is similar to Thongmee and Sukplang, (2024) report. Thus, inoculum levels above certain levels lead to lower metabolic activity and it is important that the inoculum concentrations be optimized for optimum growth and degradation.

For the growth of glufosinate ammonium-degrading bacteria, most researchers use mineral media constituted by phosphates, sulphates, and chlorides necessary for

their metabolism and supplemented with different carbon and nitrogen sources to enhance degradation (Jahan *et al.,* 2025). In this experiment Glufosinate ammonium concentration ranges between 10 to 200mg/L was used to study their effects on bacterial growth for the *Bacillus* sp. Maximum growth was observed at 10mg/L for the isolate after which the bacterial growth declined rapidly (Figure 7). One-way ANOVA identified a significant overall difference between the initial glufosinate ammonium concentrations of *Bacillus sp* ($F(5.12) = 3801.810$, $p < 0.001$). Post-hoc test for *strain* GA3 indicated that there were significant differences between the optimum initial glufosinate ammonium concentration 10 mg/L ($M = 0.3146$, $SD = 0.0007$) and the remaining glufosinate ammonium concentrations tested. A high concentration of substrate exerts a toxic effect on microorganisms, causing the inhibition of biodegradation by nutrient or oxygen limitation (Abou-Shanab *et al.*, 2012). This finding is similar to the work of Thongmee and Sukplang, (2024) that reported *Pseudomonas sp* had an optimum initial glufosinate ammonium concentration of 10 mg/L.

Nitrogen source in the medium influences the yield of the enzyme by the microorganisms. Microorganisms' preference for nitrogen is attributed to the presence of minerals, vitamins or growth factors (Fareed *et al*., 2017). Different nitrogen sources used in this experiment, highest growth was observed with glutamic acid for *Bacillus sp* (Figure 11). One-way ANOVA identified a significant overall difference between the different nitrogen sources of both *Bacillus* spp ($F(7,16) = 118078.464$, $p < 0.001$). Post-hoc test for *Bacillus sp* strain GA3 indicated that there were significant differences between glutamic acid ($M = 0.8466$, $SD = 0.00073711$) and the remaining nitrogen sources tested. This result aligns with findings of Duc (2022). who reported, glutamic acid was the best Nitrogen source.

Glutamic acid concentrations range from 0.5, 1, 1.5, 2, 2.5 and 3.0g/L. With the optimum for isolate GA3 being 1g/L as shown on Figure 12 which implies that the bacterial isolate grows best at that glutamic acid concentration of 1g/L above which it seems to have an inhibitory effect on the organisms. One-way ANOVA identified a significant overall difference between the time periods of *Bacillus sp* ($F(5, 12) = 1013.018$, $p < 0.001$). Post-hoc test for *strain* indicated that there were significant differences of bacterial growth mean values of the optimum 1 g/L ($M = 0.8607$, $SD = 0.0010$) and all the glutamic acid concentrations.

Carbon sources can influence bacterial growth rates, metabolic pathways, and biofilm formation. Optimal growth often depends on the specific carbon source and its concentration. In this study the effects of different carbon source on bacterial growth and glufosinate ammonium degradation was studied. Highest growth was observed with sucrose carbon source for the isolate GA3 as shown in Figure 13. One-way ANOVA identified a significant overall difference between the different carbon sources of *Bacillus sp* ($F(6, 14) = 8316.523$, $p < 0.001$). Post-hoc test for *strain* GA3 indicated that there were significant differences between Sucrose ($M = 0.4619$, $SD = 0.0013$) and the remaining carbon sources tested. This finding aligns with several studies that emphasize the importance of readily metabolizable carbon sources for microbial activity. For instance, research has shown that monosaccharide like sucrose is often preferred by microorganisms due to their ease of uptake and direct entry into central metabolic pathways, providing a rapid source of energy and carbon for growth (Duc*,* 2022). This enhanced metabolic activity, fueled by sucrose, likely leads to a greater production and secretion of enzymes, which are responsible for breaking down the glufosinate ammonium. This positive correlation between bacterial growth and degradation is a common observation in studies involving microbial degradation of complex substrates, where a larger and more active microbial population generally results in higher metabolic activity (Thongmee and Sukplang*,* 2024).

Figure 14 illustrates the impact of varying sucrose concentrations (0.5 – 3.0 g/L) on bacterial growth (OD600nm). The results indicate that optimal bacterial growth was achieved at a sucrose dosage of 2g/L. Below this concentration, bacterial growth was limited, likely due to substrate scarcity. Conversely, sucrose concentrations exceeding 2g/L led to a decline in bacterial growth, potentially due to substrate inhibition or an imbalance in the carbon-to-nitrogen ratio within the culture medium (Fareed *et al.,* 2017). One-way ANOVA identified a significant overall difference between the sucrose concentrations of both isolate *Bacillus sp* ($F(5, 12) = 9021.244$, $p < 0.001$). Post-hoc test for *Bacillus sp* indicated that there was significant difference of bacterial growth mean value of the optimum sucrose concentration 2g/L ($M = 2.010$, $SD = 0.0032$) to all the other concentrations.

The plackett burman design (PBD) was employed as a screening tool to identify the most significant variables from a larger set of potential factors. This approach, as highlighted by Plackett and Burman (1946) in their seminal work, allows for the evaluation of *N-1* factors in *N* experiments, offering a highly efficient method for initial variable selection. In this study, the PBD analysis of variance result (Table 2) revealed distinct sets of significant factors for bacterial growth. For bacterial growth of GA3, temperature, carbon source, and Nitrogen source. The high F-value and low p-value for these factors in the ANOVA tables (Tables 3), along with the significant model p-values ($p = 0.0421$ for GA3) and high R^2 values (0.9998 for GA3), indicate that the PBD models adequately described the relationship between the tested factors and the responses. The identification of significant factors through PBD effectively narrowed down the variables requiring further optimization, thus increasing the efficiency of the subsequent optimization phase.

Following the factor screening by PBD, the significant variables were further optimized using the CCD. CCD is a widely used second-order design suitable for fitting quadratic response surfaces and identifying optimal factor levels (Yakassai *et al.,* 2020, Babandi *et al*., 2021). The high R^2 values obtained for the CCD models for both bacterial growth of *Bacillus sp* strain GA3 (R^2 = 0.9969) demonstrate an excellent fit of the models to the experimental data. These values, being close to 1, indicate that a large proportion of the variability in the responses could be explained by the models, allowing for reliable prediction of the optimal conditions. The CCD experiments (Table 4) explored the interactions and quadratic effects of the significant factors, enabling the determination of the optimal levels for maximizing the bacterial growth of isolate GA3. While the specific optimal values derived from the CCD are presented in the results, the high R^2 values indicate the effectiveness of CCD in refining the process parameters beyond the initial OFAT optimization ANOVA CCD GA3 Table 6 shows the effective p and F values, confirming the reliability of the ANOVA-analyzed proposed model ($f = 356.32$, $p<0.0001$). These values are crucial for evaluating the validity of linear terms (A, B, C), interaction terms (AB, BC, AC), and quadratic terms (A2, B2, C2) within the model. Notably, significant p-values for A, B and C are <0.0001, 0.0019 and <0.0001, respectively. The difference between R^2 predicted bacterial growth and R^2 adjusted bacterial growth is within 0.013, affirming the suitability of the model

The plot in Figure 16 displays the similarity of actual and predicted values for Bacterial growth of isolate GA3. The actual values represent the data obtained from experiments, while the projected values are generated using the RSM in grouping with the CCD model. Both data sets exhibit a significantly high degree of proximity, underscoring the suitability of the suggested model. The R^2 values of 0.9907 for bacterial growth of GA3 indicate a strong correlation between the actual and expected responses.

The three-dimensional (3D) plots in Figures 17 visually illustrate the relationship between Bacterial growth and various process factors of isolate GA3. These plots depict similar trends, showing how bacterial growth varies with different combinations of process factors.

In this RSM-optimized study, Bacterial growth (GA3) was boosted from OD600nm (0.48) through OFAT, to OD600nm (0.91) through RSM.

Figure 18 illustrates the varied effects of different metal ions (Fe, Zn, Cd, Mn, Cu, Hg and Pb) on bacterial growth compared to the control group. Mn, Hg, Cu, Fe, Cd and Pb significantly inhibited bacterial growth of GA3, likely due to their toxic interference with essential cellular processes, enzyme function, and cell membrane integrity (Abdel-Mongy *et al.*, 2021), consequently leading to the lowest observed bacterial growth rates. Conversely Zn appeared to slightly enhance bacterial growth of GA3 suggesting a potential role as a micronutrient at the tested concentration. One-way ANOVA identified a significant overall difference between the different heavy metals of both *Bacillus sp* ($F(9, 20) = 1054.847$, $p< 0.001$). Post-hoc test for *strain* GA3 indicated that there were not significant differences of bacterial growth of control ($M = 0.3800$, $SD = 0.0093$) but there was significant difference between the control and the remaining heavy metals tested. These finding reveal the significant and often detrimental impact of heavy metal contamination on microbial bioremediation processes, highlighting the need to carefully consider the presence and concentration of specific metal ions when optimizing the degradation of glufosinate ammonium. While heavy metals like can severely impede the process, other metals like Fe, Mn, Cu, and Zn might, at specific concentrations, have neutral or even slightly beneficial effects on glufosinate ammonium-degrading bacteria (Usman *et al.,* 2023).

The measurement of glufosinate ammonium degradation depicted in Figures 19 showing the peak area of control, isolate GA3. The peak integration (Trapezoid method) enables the determination of the reduction in the intensity of glufosinate ammonium in the sample as it was directly proportional to the concentration. Isolate GA3 degradation rate of glufosinate ammonium was calculated to be 75.9%.

CONCLUSION

This study successfully isolated and identified indigenous glufosinate-ammonium degrading bacteria from agricultural soils with notable potential for herbicide degradation, through morphological, biochemical resulted to gram positive and molecular (16S rRNA) characterization, the isolate GA3 was confirmed to belong to genera *Bacillus sp*. Optimization of degradation conditions using the One-Factor-At-a-Time (OFAT) resulted to temperature 350C, pH 7.0, inoculum size 5%, substrate concentration 10mg/L, nitrogen source glutamic acid 1g/L, carbon source sucrose 2g/L, and Response Surface Methodology (RSM) proved that the bacterial isolate GA3 was able to utilized glufosinate ammonium as a nutrient source, This dual-optimization strategy enabled the determination of statistically significant parameters influencing degradation efficiency at 75.9% when experiment was validated using LC-MS. *Bacillus* strain obtained exhibited high degradation rates, making it potential for glufosinate ammonium bioremediation in the soil.

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