Streptomyces sp. Strain SRH22: A Potential Bioremediation Agent for Glyphosate-Contaminated Agricultural Soils

Hadjer Rebai[1,2*], Essam Nageh Sholkamy[3], Reem Mohammed Alharbi[4], Neveen Abdel-Raouf [5,6], Oumeima Boufercha[1], Paula Castro[7], and Allaoueddine Boudemagh[1]**

[1]*Laboratory of Molecular and Cellular Biology, Constantine 1- Frères Mentouri University, Chaâbat Erssas Campus, Ain El Bey Road, 25000 Constantine, Algeria*
[2]*Department of Microbiology, Constantine 1- Frères Mentouri University, Chaâbat Erssas Campus, Ain El Bey Road, 25000 Constantine, Algeria*
[3]*Department of Botany and Microbiology, College of Science, King Saud University, Saudi Arabia*
[4]*Biology Department, Science College, University of Hafr Al Batin, Hafr Al Batin, Saudi Arabia*
[5]*Department of Biology, College of Science and Humanities in Al-Kharj, Prince Sattam Bin Ab-Dulaziz University, Saudi Arabia*
[6]*Department of Botany and Microbiology, Faculty of Science, Beni-Suef University, Salah Salem Street, Beni-Suef 62511, Egypt*
[7]*CBQF-Centro de Biotecnologia e Química Fina-Laboratório Associado, Escola Superior De Biotecnologia, Universida De Católica Portuguesa, Portugal*

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*** Corresponding author:**
E-mail: hadjer.rebai@umc.edu.dz

**** Corresponding author:**
E-mail: essam_92003@yahoo.com

ABSTRACT

Glyphosate, also known as N-phosphonomethylglycine, is the herbicide that is widely used across the globe. As there are concerns over its potential toxicity to non-target soil species, there is a growing interest in identifying glyphosate-degrading microorganisms in soil. Biodegradation, by actinobacteria, is a very promising approach to eliminate this pesticide from contaminated environments. The present work isolated and identified actinobacteria capable of degrading glyphosate from Saharan agriculture, as well as determined how the application of this herbicide affects the abundance of actinobacteria present in soil. It was observed that the use of glyphosate led to an increased abundance of actinobacteria in the soil compared to the untreated soil. Among this population, an actinobacterial strain was isolated from glyphosate contaminated soil by the enrichment method, and was identified to possess the greatest capability to degrade glyphosate at 50 mg/L. The identification of this strain was achieved through a combination of cultural, morphological, biochemical, and molecular techniques. This included the use of 16S rDNA sequencing, leading to its successful classification as *Streptomyces* sp. strain SRH22. This strain was assigned the accession number OQ302556 by the National Center for Biotechnology Information (NCBI). A rapid, sensitive, and straightforward spectrophotometric technique was employed for the quantification of glyphosate. Results showed that the optimal biodegradation (90.2%) was obtained under a temperature of 30 degrees, a PH of 7.2, and an inoculum volume of 4% timed over six days. This work shows that the *Streptomyces* SRH22 presents good potentiality to be used as a bioremediation agent for agricultural soils in the Algerian Sahara.

1. INTRODUCTION

The use of pesticides is an important and necessary practice in agriculture due to their ability to kill pests and reduce crop diseases, primarily for economic reasons (Sabzevari et al., 2022). Pesticides are widely used throughout the world, including in Saharan agronomy in Algeria (Supreeth et al., 2016; Arias-Estévez et al., 2008; Belhadi et al., 2016) However, the use of pesticides in agriculture can have severe environmental consequences, including air, soil, and water pollution. Exposure to these chemicals can also harm non-target organisms, leading to fatalities from accidental poisoning. Among the most dangerous pesticides, we found organophosphates

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(OPs), which are extensively used and known to persist in the environment for long periods (Jiang et al., 2019; Briceño et al., 2018; Cardozo et al., 2019).

Glyphosate, commonly known as N-phosphono-methyl-glycine, is an extensively used herbicide in the Sahara Region of Algeria. It is an organophosphate pesticide that targets weeds by inhibiting the activity of the enzyme EPSPS which affects the production of amino acids, reducing the level of these essential compounds (De Castilhos Ghisi et al., 2020; Maeda and Dudareva, 2012). Glyphosate is the active ingredient in the commercial product Fortin SL (Zhan et al., 2018), which has been registered and sold in Algeria for years and used in agriculture such as vegetable crops, citrus orchards, and palm groves in the Saharan agricultural soils of Algeria. Recently, the European Union authorized its use until December 15th, 2023.

Glyphosate continues to be used in Algeria, and its half-life in the environment ranges from 0.8 to 151 days. This is influenced by variation in environmental conditions and soil type (Bai and Ogbourne, 2016). Reports from experts suggest that the harmful residues of this herbicide can accumulate in soil and water, posing a potential environmental hazard (Firdous et al., 2020). To eliminate this pesticide, bioremediation has been identified as the most effective, and economical, mechanism which involves the use of microorganisms to restore various environmental sites that have been negatively impacted (Manogaran et al., 2017; Uqab et al., 2016; Briceño et al., 2017; Mishra et al., 2021; Rossi et al., 2021).

Actinobacteria, a type of Gram-positive bacteria with a filamentous structure, are abundant in soil and have remarkable abilities to degrade xenobiotic compounds such as pesticides (Alvarez et al., 2016). While several studies have shown that actinobacteria can degrade different chemical classes of pesticides, including organochlorines such as lindane, y-chlordane, and methoxychlor (Fuentes et al., 2017), most reports on glyphosate biodegradation have focused on microorganisms other than actinobacteria (Manogaran et al., 2017; Fuentes et al., 2017; Hadi et al., 2013; Adelowo et al., 2014; Malla et al., 2023; Yu et al., 2023).

Due to the enzymatic diversity of actinobacteria, and the lack of research on the biodegradation of glyphosate in arid soils by these bacteria, we sought to explore their metabolic potential in this area. Our main focuses are on the isolation and identification of actinobacteria that could degrade glyphosate, a commonly used herbicide in Algerian agriculture. Additionally, we aimed to assess the potential of this strain in mitigating the negative effects of glyphosate on the actinobacterial population in contaminated soil.

2. METHODOLOGY

2.1 Geographic location

The study was carried out in the city of Ouargla, situated in the northeast of the northern Sahara, which is located about 750 km to the south of Algiers.

Ouargla is bordered by the wilaya of El-Oued to the northeast; the wilaya of Djelfa to the northwest; the wilaya of Illizi to the southeast and by the wilaya of Ghardaïa to the west (Abdelhak, 2020).

2.2 Effect of glyphosate on the number of actinobacteria

To evaluate the impact of glyphosate herbicide on the actinobacteria population, in arid soil of Ouargla, two soil samples were examined: one treated with glyphosate and another untreated. The selective GLM and Bennett medium were used to count the actinobacteria, and their numbers were determined by applying a serial dilution technique. The soil was first mixed with distilled water to create a stock solution, which was then diluted several times to create a series of dilutions up to 10^{-5}. Next, from each dilution, an amount of 0.1 mL was inoculated onto the nutrient medium and incubated at a temperature of 30°C, for 21 days to allow for bacterial growth and enumeration.

2.3 Isolation and identification of actinobacteria

Isolation by enrichment: Actinobacteria were isolated from agricultural soil located in Ouargla City, Algeria, through an enrichment method using Vendermesse's minimum medium (MSM) with 50 mg/L of glyphosate as a carbon source, according to the protocol of Abraham and Gajendiran (2019).

2.4 Glyphosate tolerance of actinobacterial isolates

Following the protocol of Briceño et al. (2012), several actinobacterial isolates were used to determine their ability to degrade glyphosate pesticide at varying concentrations (1, 10, 25, and 50 mg/L). Based on the results, one of the actinobacterial isolates was identified as having a high potential for biodegrading glyphosate pesticide.

2.4.1 Morphological, biochemical and physiological characterization

The selected actinobacterial isolate was subjected to a study of its macro and micro morphological characteristics. The isolate was streaked on various nutrient media, including ISP2, ISP7, ISP9, GLM, Bennett, and YEMA, then incubated during a week at 30°C to observe macroscopic characteristics. Following this, Gram staining and spore morphology were examined using a light microscope. To evaluate the bacterial isolate's ability to use different sugars as a carbon source, such as D-fructose, galactose, glucose, lactose, sachharose, mannitol, and citrate (Shirling and Gottlieb, 1966). In addition, various amino acids (aspartic acid, laproline, arginine, threonine, histidine, asparagine, tyrosine, and methionine) were tested as a nitrogen source (Williams et al., 1983). Other tests conducted included catalase production (Li et al., 2016), gelatin hydrolysis (Minotto et al., 2014), starch hydrolysis (Tatsinkou Fossi et al., 2005), and melanoid pigment production (Lee et al., 2014). The strain's tolerance to different pH levels (2, 5, 9, 12), temperatures (4°C, 28°C, 37°C, 40°C, 50°C), and NaCl concentrations, ranging from 2% to 15%, were also tested using ISP2 medium.

2.4.2 Amplification of 16S ribosomal RNA gene and nucleotide sequencing through polymerase chain reaction (PCR)

DNA extraction was performed using the DNeasy Power Soil kit. A pair of forward (27F 5′AGAGTTTGATCCTGGCTCAG-3′) and reverse (1429R 5′-GGTTACCTTGTTACGACTT-3′) primers were used for the PCR reaction. The denaturation of target DNA was done at 95°C for 5 min, followed by amplification with 30 cycles at 94°C for 1 min, then, at 55°C for 1 min, and at 72°C for 1.50 min. The PCR mixture was then maintained at 72°C for 10 min (Boufercha et al., 2022). The amplified PCR products were purified and sequenced at Eurofins genomics (Konstanz-Germany) using universal bacterial RNA16S primers (27F) (Moreira et al., 2021). Identification was performed using the BLAST software. Phylogenetic analysis was conducted by aligning the bacterial 16S rRNA gene sequences with reference sequences available in the GenBank database. The Neighbor-Joining method was implemented using the MEGA software (version 11) to construct the phylogenetic tree.

2.5 Growth kinetics

To record the growth curve of the actinobacterial strain, the protocol of Briceño et al. (2012) was used with simple modification. Briefly, 4% of the bacterial pellet was cultivated in liquid MSM medium, with 50 mg/L of glyphosate as the sole carbon source, and placed on a shaker at 100 rpm at 30°C for 10 days.

2.6 Biodegradation of glyphosate by action-bacterial isolate

To evaluate the rate of glyphosate biodegradation, a spectrophotometric technique proposed by Bhaskara and Nagaraja (2006) was used. 30 mL of MSM medium, with glyphosate at 50 mg/L, were prepared in 50 mL erlens and inoculated with an actinobacterial isolate pellet at a concentration of 4%. Abiotic controls were also prepared, followed by incubation, during a ten day period at 30°C under 200 rpm. 1 mL of the culture was taken every day and passed to centrifugation at 6,000 g for 10 min at room temperature. It was then filtered through a PVDF polyvinylidene fluoride membrane filter of 0.22 µm. The filtered liquid was mixed with a solution of a mixture containing 0.5 mL of ninhydrin and 0.5 mL molybdate (at 5% for each solution). A standard curve was also prepared under the same experimental conditions, added by glyphosate at concentrations from 4 to 14 mg/L. The tubes were covered with aluminum foil to prevent exposure to light, and incubated at 100°C for 5 min followed by cooling at room temperature. Finally, 3 mL of distilled water was subsequently added to reach a final volume of 5 mL. The absorbance measurements were taken using a UV-visible spectrophotometer (UV-1800A, Shimadzu, Japan) at 570 nm. The experiment was carried out in triplicate, and the percentage of glyphosate biodegradation was calculated using the following formula.

$$\text{Biodegradation percentage (\%)} = (M1 - M2)/M1 \times 100\%$$

M1 and M2 represent the concentrations of glyphosate in the untreated and treated samples, respectively, using the actinobacterial isolate.

2.7 Effect culture conditions on glyphosate biodegradation

A 4% inoculum of actinobacterial isolate was introduced into 30 mL of Vendermesse's minimum

medium, which contained 50 mg/L of glyphosate. The flasks with different pH levels (2, 5, 7, 9, 12), various temperatures (4°C, 10°C, 20°C, 25°C, 30°C, 37°C, and 40°C) and various concentrations of actinobacterial inoculum (2%, 4%, 5%, 7%, and 9% w/v) were incubated at 30°C with agitation at 100 rpm/min for 10 days. During the incubation period, the glyphosate concentration was measured every 24 h (Bhaskara and Nagaraja, 2006).

2.8 Statistics analysis

All experimental procedures were performed in triplicate with standard error. GraphPad Prism version 8.0 (GraphPad Software, Inc., La Jolla, CA, USA) was used to carry out statistical analyses. ANOVA analyses (one-way and two-way) of variance and Tukey's test were utilized with a significance level of $p \leq 0.05$ (95% confidence interval).

3. RESULTS

3.1 Effect of glyphosate on the number of soil actinobacteria

Actinobacteria isolated from herbicide-treated and untreated soil samples, on both GLM and Bennett media, were recognised by their characteristic aspects and then enumerated. Their numbers in the untreated soil were 220 CFU/mL in the GLM medium and 300 CFU/mL in the Bennett medium. On the other hand, in the treated soil, the number was 500 CFU/mL in the GLM medium and in the Bennett medium, it was 300 CFU/mL (Figure 1).

3.2 Morphological characteristics of action-bacterial isolate SRH22

One strain among the eight actinobacterial isolates was selected for this study based on its superior tolerance to the highest glyphosate concentration 50 mg/L. This actinobacterium SRH22 showed a circular colony morphology, with a pasty texture and firm adherence to the agar, with yellow substrate mycelium, grey aerial mycelium and Gram-positive. The shape of spore chains was Retinaculum-Apertum. When it was grown on ISP2 medium and ISP7, the isolate produced yellow pigments. The isolate showed good growth on ISP2, GLM, Bennett, and YEMA media (Figure 2 and Table 1).

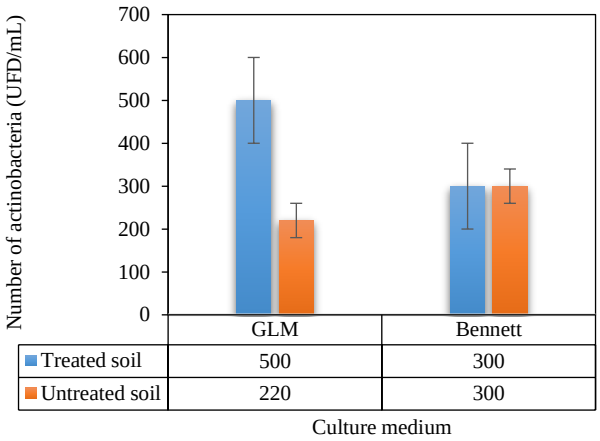

Figure 1. Determination of number of actinobacteria in treated and untreated soil with Glyphosate pesticide.

3.3 Biochemical and physiological characteristics of the actinobacterial isolate SRH22

The actinobacterial isolate SRH22 showed positive hydrolysis of starch, gelatin and casein, as well as the ability to produce catalase, coagulate and peptonize milk. In addition, negative results were observed on Nitrate reductase, RM, VP, H_2S and mobility. The isolate SRH22 used glucose, D-fructose, and galactose as a source of carbon, and lactose, saccharose, citrate, and mannitol were not utilised. All tested nitrogen sources, including aspartic acid, proline, arginine, threonine, histidine, asparagine, tyrosine, and methionine, were utilized by the isolate. SRH22 showed moderate growth on media containing 2% and 5% sodium chloride, poor growth on 9%, and no growth on 15% NaCl. It was capable of growing across the pH range tested (2, 5, 7, 9, and 12), with optimal growth at pH 7. The isolate showed strong growth at 28°C and 37°C, moderate growth at 40°C, weak growth at 4°C and no growth at 50°C (Table 2).

Table 1. Cultural characteristics of *Streptomyces* sp. isolate SRH22 after incubation during 15days at 30°C in different nutrient media.

Medium	Growth	Aerial mycelium	Substrate mycelium	Form of spores chain	Pigmentation
ISP-2	Strong	Strong, gray	Present	Retinaculum-Apertum	Yellow
ISP-7	Moderate	Weak, gray	Present		
ISP-9	Moderate	Weak, gray	Present		
GLM	Strong	Strong, gray	Present		
Bennett	Strong	Weak, gray	Present		
YEMA	Strong	Weak, gray	Present		

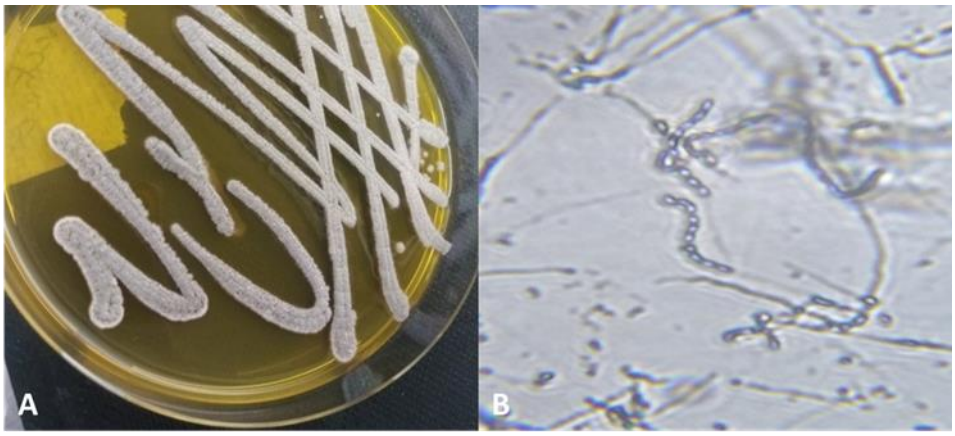

Figure 2. (A) Morphological image of the actinobacterial isolate SRH22 growth on ISP-medium after 7 days; (B) Microscopic observation of the isolate SRH22 (G.100)

Table 2. Biochemical and physiological characteristics of the actinobacterial isolate SRH22

Enzyme activity	Result
Production of H2S	-
Reaction RM	-
Reaction VP	-
Nitrate reduction	-
Catalase	+
Starch hydrolysis	+
Gelatin hydrolysis	+
Casein hydrolysis	+
Coagulation of skim milk	+
Peptonization of skim milk	+
Utilisation of nitrogen sources	**Result**
Aspartic acid	+
The proline	+
Arginine	+
Threonine	+
Histidine	+
Asparagine	+
Tyrosine	+
Methionine	+
Utilisation of carbon sources	**Result**
Glucose	+
Galactose	+
D-Fructose	+
Lactose	-
Saccharose	-
Mannitol	-
Citrate	-
Growth at NaCl %	**Result**
2%	++
5%	++
9%	+
15%	-
Growth at different pH	**Result**
2	++
5	++
7	+++
9	++
12	++
Growth at different temperatures	**Result**
4	+
28	+++
37	+++
40	++
50	-

3.4 Molecular identification

The phylogenetic tree was created by comparing the actinobacterial isolate's 16S rRNA gene sequence, which contained 948 nucleotide bases, with 18 *Streptomyces* 16S rRNA gene sequences from NCBI databases. The actinobacterial isolate presented 88% to 99% similarity with *Streptomyces* species such as *Streptomyces ambofaciens* strain S8-36 (accession no. MW339011), *Streptomyces humiferus* JCM 3037 (accession no. MT760387), *Streptomyces violaceoruber* strain EA128 (accession no. MW642118), *Streptomyces marrokonensis* strain 2 (accession no. MW695204), and *Streptomyces tricolor* strain CIAD-CA43 (accession no. MK96859). According to the phylogenetic tree analysis, our actinobacterial isolate is closely related (88%) to *S. coelicolor* sp. strain A3 (accession no. OP315308), as shown in Figure 3. Based on the morphological, molecular, and phylogenetic analyses, the action-bacterial isolate was identified as *Streptomyces* sp. strain SRH22 under Accession No OQ302556.

3.5 Growth kinetics of SRH22 isolate in liquid medium

The actinobacterial isolate SRH22's growth on liquid MSM medium, supplemented with 50 mg/L glyphosate as a carbon source, was determined by monitoring the amount of cell dry mass in mg/mL (Figure 4). The isolate showed good growth, without any lag phase, for the first 24 h, and the logarithmic phase persisted until the 6th day when the isolate reached its growth optimum of 0.382 mg/mL. The growth rate remained unchanged thereafter. In comparison, the actinobacterial growth exhibited significant variation (p-value=0.0022). The biotic control, which represents the culture without pesticide, was also included in the study.

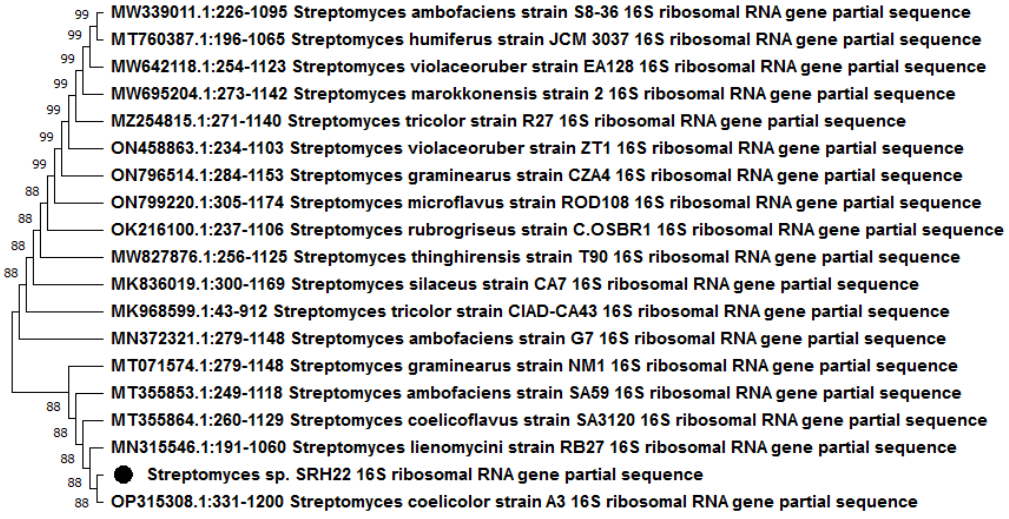

Figure 3. Phylogenetic tree analysis of *Streptomyces* sp. strain SRH22

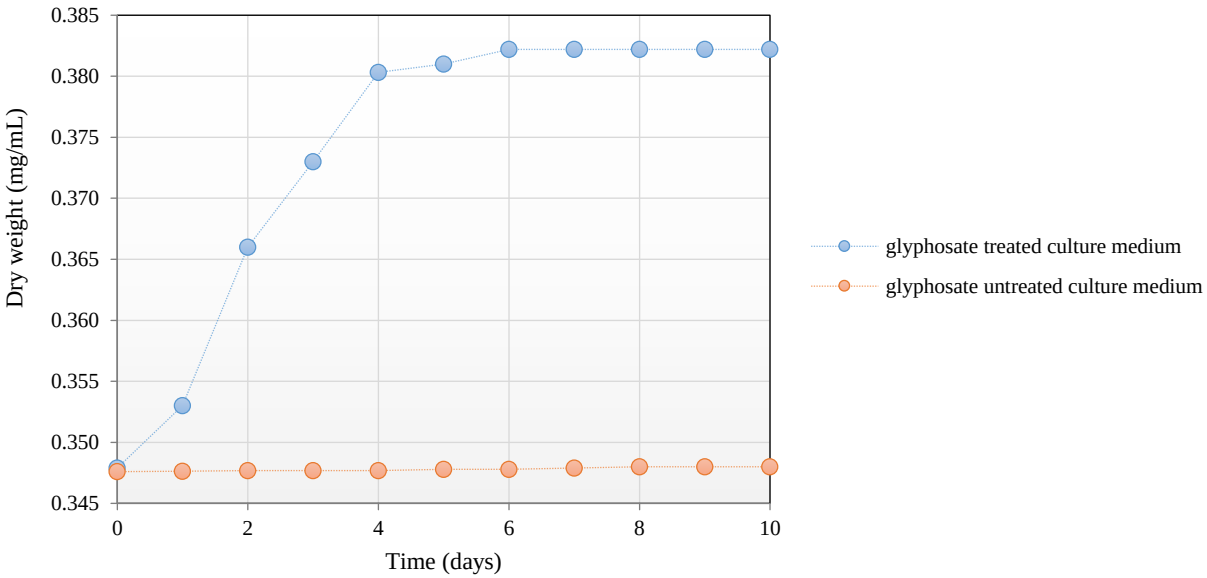

Figure 4. Growth kinetics of strain SRH22 with glyphosate at the concentration 50 mg/L

3.6 Kinetics of glyphosate biodegradation

The degradation of glyphosate, by the isolate SRH22, was demonstrated by the disappearance of the purple color that indicates the C-N bond between glyphosate and ninhydrin, while the control tube retained the purple color. Within the first 24 h, the isolate SRH22 exhibited an estimated percentage of glyphosate degradation of 74%. Over the next 6 days, degradation rates increased with time and reached a maximum degradation capacity of 90.2%, which showed significant differences (p-value<0.0001). After 6 days of incubation, the percentage of degradation became stable for the remaining days (Figure 5).

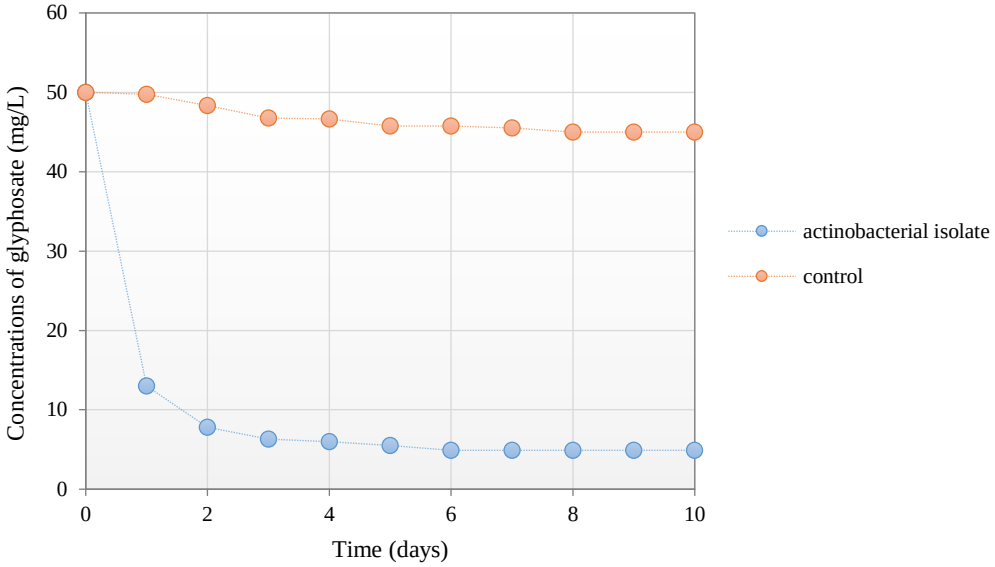

Figure 5. Determination of the quantity of the degraded glyphosate by *Streptomyces* sp. strain SRH22

3.7 Effect of culture conditions on glyphosate biodegradation

3.7.1 Effect of pH on glyphosate biodegradation

Figure 6 showed the effect of pH on glyphosate biodegradation. The highest percentage of degradation was observed at pH 7.2, after 6 days of culture, which amounted to 90.2%. The lowest biodegradation rate was marked at acidic pH 2 and 5 with the percentage of 14.59% and 18.36% respectively. At Basic pH, 9 and 12, the strain SRH22 removed 32.88% and 30.35% respectively. However, at neutral pH 6.5 and 7.5, good degradation was observed reaching 58.24% and 69.97% respectively. The independent variance pH had a significant impact on the glyphosate degradation percentage, with an increase from pH 2 to 7.2 causing the degradation rate to increase from 14.59% to 90.2%. However, there was no significant difference in glyphosate biodegradation from the 5th day when the pH was ≤7±0.2 and from the third day when the pH was ≥7±0.2 (p-value>0.05).

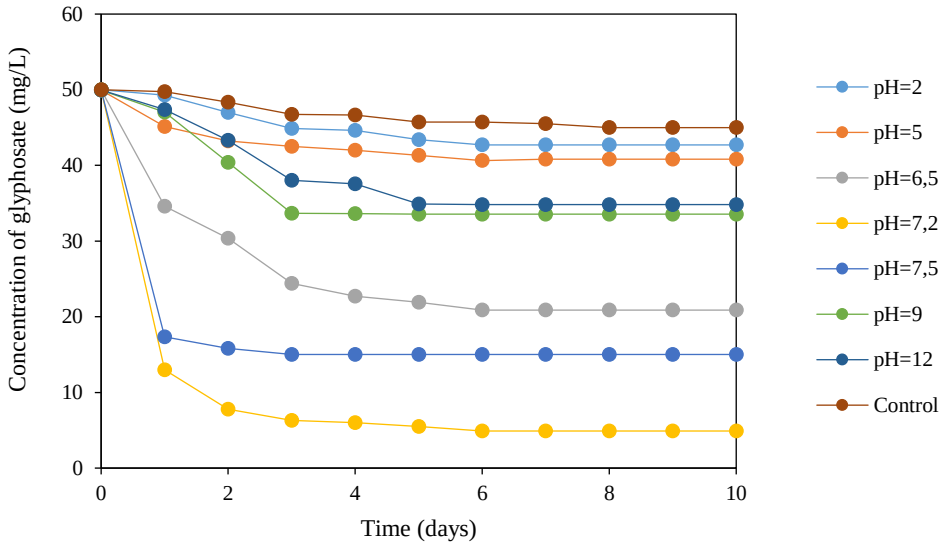

Figure 6. Effect of pH on glyphosate biodegradation by *Streptomyces* sp. strain SRH22

3.7.2 Effect of temperature on the bio-degradation of glyphosate

The results of glyophosate degradation, under different temperatures, indicate that the most efficient temperature was 30°C with 90.2% of glyphosate elimination during 6 days of incubation (Figure 7). The results showed that the percentage of degradation was proportional to the increase in temperature.

SRH22 bacteria was able to degrade 14.18%, 19.86%, 37.16%, 45.17%, and 68.87% at temperatures of 4°C, 10°C, 20°C, 25°C, and 37°C respectively. However, at temperature 40°C, a decrease in the rate of biodegradation was observed as 43.80%. These findings suggest that temperature is a crucial factor that impacts the degradation rate of glyphosate. The effect of temperature on glyphosate biodegradation was significant at temperatures ranging from 4°C to 30°C, over an interval of 3 to 6 days. Additionally, with an increase in temperature from 37°C to 40°C, the effect was significant over an interval of 1 to 2 days with a p-value of less than 0.001.

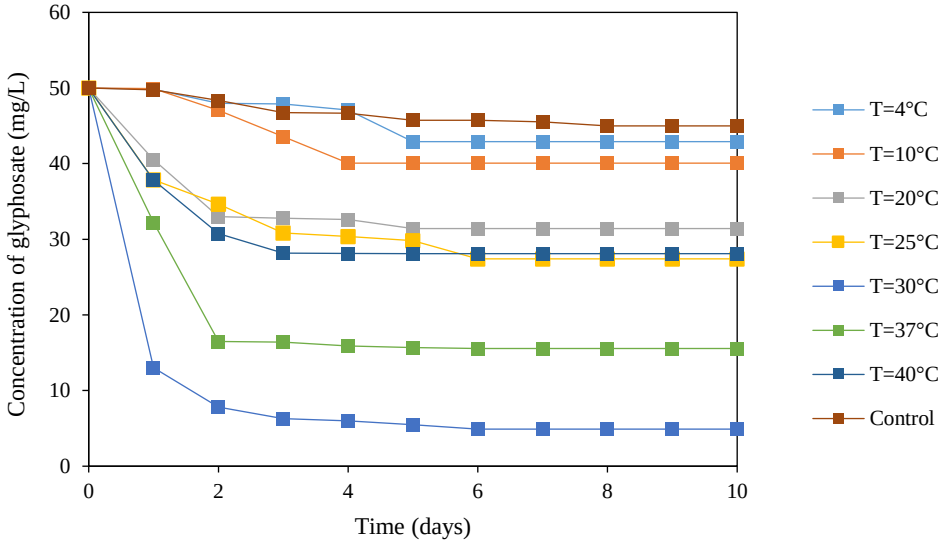

Figure 7. Effect of temperature on glyphosate biodegradation by *Streptomyces* sp. strain SRH22

3.7.3 Effect of inoculum on glyphosate biodegradation

The results of glyphosate degradation, with different inoculum size, indicate that the highest biodegradation rate of 90.2% was observed within 6 days with inoculum volume 4%. During the first six days of incubation and at inoculum volumes of 2%, 5%, 7%, and 9%, the degradation progressively decreased to 17.31%, 89.32%, 38.82%, and 32.72% respectively (Figure 8). The inoculum size has an impact on glyphosate degradation by the SRH22 strain. An inoculum size of 4% resulted in a high percentage of degradation (90.2%), while inoculum sizes smaller or larger than the optimal 4% had a negative effect on degradation. The results suggest that the impact of the inoculum size on glyphosate degradation was significant (p-value<0.001).

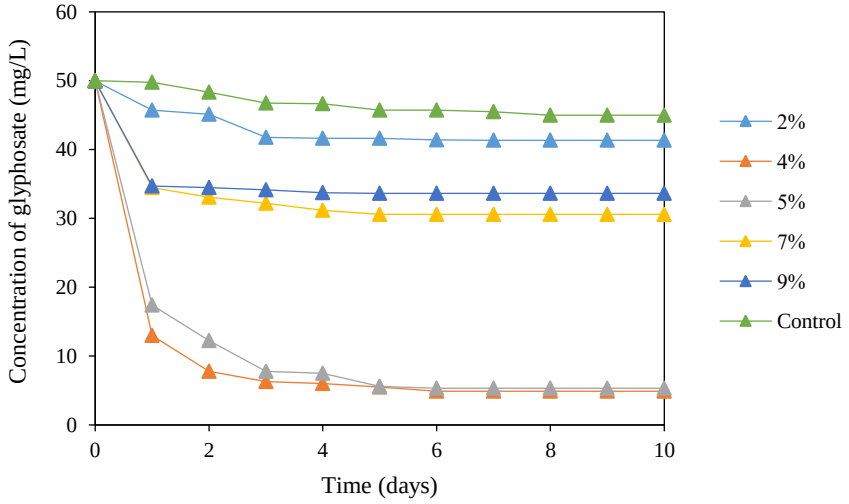

Figure 8. Effect of different inoculum volumes on glyphosate degradation by *Streptomyces* sp. strain SRH22

4. DISCUSSION

The use of herbicides in agriculture in Ouargla, a region in the Algerian Sahara, has led to the accumulation of pollutants in the soil. This poses a significant challenge for soil remediation in these arid land ecosystems (Sviridova et al., 2015; Benslama and Boulahrouf, 2013). The biological approach to soil remediation is considered more ecologically friendly and less expensive than physical-chemical techniques (Zhan et al., 2018). Glyphosate is a widely used herbicide efficiently eliminated by microorganisms (Bhatt et al., 2021a), Over time, various studies have confirmed the ability of different microorganisms to degrade glyphosate (Abosereha et al., 2022; Elarabi et al., 2020; Ermakova et al., 2017). However, studies on the biodegradation of glyphosate by the genus *Streptomyces* are rare (Singh et al., 2019; Lipok et al., 2009; Obojska et al., 1999), although this particular genus of *actinomycetales* being the most dominant in soil, accounting for more than 95% of the identified bacteria (Barka et al., 2016).

Several works have isolated different species of the genus *Streptomyces* from arid soils, which offers a promising prospect for treating these contaminated areas with this bacterial genus (Boudemagh et al., 2005; Korayem et al., 2015; Reghioua et al., 2006; Souagui et al., 2015). In this context, *Streptomyces* sp. SRH22 has been identified as a potential bioremediation agent for glyphosate-contaminated agricultural soils in the Algerian Sahara. The strain SRH22 was found to effectively use glyphosate as the sole source of carbon.

The herbicide glyphosate showed a positive impact on the actinobacterial population in treated soil, with an increase in their numbers compared to untreated soil with glyphosate. This result is consistent with findings from other researchers who have shown that when glyphosate is used in soil it becomes soluble and inactive, losing its antimicrobial effect. This encourages soil microorganisms to use it as an energy and nutrient source (Prankle et al., 1975; Kuklinsky-Sobral et al., 2005). Similarly, Araujo et al. (2003) observed a significant increase in the population of actinobacteria in soil in the presence of glyphosate. However, other studies suggest that glyphosate in soil can stimulate or inhibit soil microorganisms, depending on the pesticide type and soil characteristics (Carlisle and Trevors, 1986; Subhani et al., 2000).

In this study, among eight isolated strains, only one actinobacterium showed the highest tolerance to various tested concentrations of glyphosate. According to the cultural, macroscopic, and microscopic characteristics, and 16S rRNA identification, the isolate was named *Streptomyces* sp. SRH22. The SRH22 were found to use glyphosate at 50 mg/L as the sole source of carbon in MSM liquid medium, which is the case for the majority of microorganisms that use glyphosate as a source of nutrients for their growth (Hernandez Guijarro et al., 2018).

Glyphosate degradation was quantified using the UV-spectrophotometric method, proposed by Bhaskara and Nagaraja. (2006), which is simple, cost effective, and easy to use in developing countries (Nnamonu and Nkpa, 2012). In this reaction, glyphosate reacts with ninhydrin in the presence of molybdate to form a blue-purple complex. The intensity of the color produced is proportional to the amount of glyphosate present in the sample (Xu et al., 2018).

The *Streptomyces* sp. SRH22 showed direct growth during the first 24 h of incubation, without any adaptation period, utilising approximately 74% of glyphosate as a carbon source. This result could be attributed to the adaptation of the actinobacterium to the herbicide, resulting in an efficient degradation metabolism. Similar results have been observed in other studies involving the degradation of glyphosate by the strain *Chryseobacterium* sp. Y16C (Zhang et al., 2022) and the degradation of Lindane, y-chlordane, and methoxychlor by the genus *Streptomyces* (Fuentes et al., 2017). After 6 days of culture, the *Streptomyces* sp. SRH22 removed 90.2% of glyphosate, and the biodegradation rate remained stable at 90.2% for 10 days. Our results are very satisfactory compared to other studies. For instance, Singh et al. (2019) reported that an actinobacterium assigned to the genus *Streptomyces* sp. used 89.77% of glyphosate after 7 days of culture. Other bacteria, such as *Bacillus subtilis* and *Rhizobium leguminosarum*, used 87.64% and 86.17% of glyphosate, respectively, after 336 h (Singh et al., 2019). Kryuchkova et al. (2013) found that the bacterium *Enterobacter cloacae* K7 was able to utilise 50% of the initial glyphosate concentration 5 mM after 5 days of incubation. The strains *Ochrobactrum* sp. B18, *Pseudomonas citronellolis* ADA-23B, *Ochrobactrum* sp. Ge-14 and *Ochrobactrum* sp. DGG-1-3 isolated by Gongora-Echeverría et al. (2020) degrade 60% of glyphosate at initial concentration 50 mg/L after 15 days of incubation. However, Zhang et al. (2022) showed a 100% degradation of glyphosate by a new isolate *Chryseobacterium* sp. Y16C in 4 days. The difference in the ability of the bacteria to degrade

glyphosate is linked to the specific characteristics of the strain (Ermakova et al., 2017).

The biodegradation of glyphosate at different pH, temperatures and inoculum volume is important to show the most favorable conditions for effective degradation of glyphosate. The strain *Streptomyces* sp. SRH22 presented an effective degradation rate, at a neutral pH 7.2, however, lowest rate of biodegradation was founded at acidic pH. This result is similar with studies of Zhan et al. (2022) who reported that the strain *Chryseobacterium* sp. Y16C degrade 100% of initial glyphosate concentration at pH range of 7-9 and, however acidic pH 5 and 6, the degradation was 73.63% and 81.08% respectivelly. Manogaran et al. (2017) found that the optimium pH for glyphosate degradation by the isolate *Burkholderia vietnamiensis* strain AQ5-12 was 6 and 7 and low biodegradation rate was observed at acidic pH of 4, 4.5. These results were expected, as most studies on glyphosate biodegradation have shown that the majority of bacteria prefer neutral or alkaline pH to eliminate glyphosate (Singh and Walker, 2006).

The Biodegradation of glyphosate, by the strain SRH22, was higher at temperature 30°C, same temperature was found by many studies such as Zhan et al. (2022), Hadi et al. (2013) by the strain *Ochrobactrum* sp. GDOS. The pH and temperature are critical factors that affect the biodegradation process of pesticides, affecting the structure of enzymes and causing their denaturation, which affects the ability of the strain to degrade the pesticide.

The highest degradation percentage was observed at an inoculum concentration of 4%, and it decreased as the inoculum percentage was either increased or decreased, which is consistent with the findings of Zhang et al. (2014). However, Nourouzi et al. (2012) found that the values of the degradation rates of glyphosate by bacteria increased with an increasing of the initial inoculum size. These findings highlight the potential of *Streptomyces* sp. SRH22 as an environmentally friendly and cost-effective alternative to traditional physical-chemical methods of soil remediation in the Algerian Sahara. Further research is needed to investigate the long-term efficacy of this bioremediation strategy and the potential development of herbicide resistance within the actinobacterial population.

5. CONCLUSION

Streptomyces sp. strain SRH22 was isolated from agricultural soil in Ouargla and demonstrated a good tolerance and ability to grow in the presence of high concentrations of glyphosate (50 mg/L) as the only source of carbon. Identification was performed using both phenotypic and molecular methods. The study determined that the most favorable conditions for glyphosate degradation were at pH 7.2, a temperature of 30°C, during 6 days, and a volume of inoculum of 4%, resulting in a 90.2% glyphosate biodegradation percentage. Additionally, the research found that glyphosate had a positive impact on the number of soil actinobacteria. These findings suggest that the *Streptomyces* SRH22 strain holds promising potential as a bioremediation agent in Saharan agricultural soils.

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