

Resistance of *Bradyrhizobium* sp. (*Lotus*) to metallic stress

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Abstract

The objective of this study is to characterize the bacterial strain *Bradyrhizobium* sp. (*Lotus*) and to determine its resistance to trace metal elements (TM) such as copper (Cu(II)) and cadmium (Cd(II)). *Bradyrhizobium* sp. (*Lotus*) was isolated from nodulated plants of *Lotus ornithopodioides*. Different characteristics of the strain were evaluated, such as growth, bacterial mobility, enzyme and polysaccharide production. The minimum inhibitory concentration (MIC) of TM was determined in vitro and in silico by measuring the optical density at a specific wavelength. The isolate is identified as *Bradyrhizobium* sp. (*Lotus*), which is a *Plant Growth Stress Regulating Bacteria* (PGSRB) Gram-negative and slow growing, producing succinoglycan, mobile, possessing urease activity. The isolate produces Indole Acetic Acid (IAA) at 70.6 µg/ml. *Bradyrhizobium* sp. (*Lotus*) showed more pronounced resistance to Cd(II) compared to Cu(II). The MIC of Cd(II) was estimated in silico to be approximately 10 gL⁻¹, while the MIC of Cu(II) was 2 gL⁻¹. Statistical analyzes confirmed a significant relationship between the concentration of TM and the number of bacterial cells. This study made it possible to characterize the bacterial strain *Bradyrhizobium* sp. (*Lotus*) and determine its resistance to TM. The results suggest that this strain can be used in remediation or bioremediation applications involving environments contaminated with heavy metals such as copper and cadmium.

Keywords : *Lotus ornithopodioides* L., Heavy metals, Phytoremediation, Rhizobacteria, PGPR

Résistance de *Bradyrhizobium* sp. (*Lotus*) aux contraintes métalliques

Résumé

L'objectif de cette étude est de caractériser la souche bactérienne *Bradyrhizobium* sp. (*Lotus*) et de déterminer sa résistance aux éléments traces métalliques (ETM) tels que le cuivre (Cu(II)) et le cadmium (Cd(II)). *Bradyrhizobium* sp. (*Lotus*) a été isolé à partir de plantes nodulées de *Lotus ornithopodioides*. Différentes caractéristiques de la souche ont été évaluées, telles que la croissance, la mobilité bactérienne, la production d'enzymes et de polysaccharides. La concentration minimale inhibitrice (CMI) des ETM a été déterminée in vitro et in silico en mesurant la densité optique à une longueur d'onde spécifique. L'isolat est identifié comme *Bradyrhizobium* sp. (*Lotus*), une bactérie régulatrice de la croissance des plantes soumises au stress (PGSRB) à croissance lente et Gram-négatif, produisant du succinoglycane, mobile et possédant une activité uréasique. L'isolat produit de l'acide indol acétique (IAA) à une concentration de 70,6 µg/ml. *Bradyrhizobium* sp. (*Lotus*) a montré une résistance plus prononcée au Cd(II) par rapport au Cu(II). La CMI du Cd(II) a été estimée in silico à environ 10 g/L, tandis que la CMI du Cu(II) était d'environ 2 g/L. Les analyses statistiques ont confirmé une relation significative entre la concentration d'ETM et le nombre de cellules bactériennes. Cette étude a permis de caractériser la souche bactérienne *Bradyrhizobium* sp. (*Lotus*) et de déterminer sa résistance aux ETM. Les résultats suggèrent que cette souche peut être utilisée dans des applications de remédiation ou de biorémédiation impliquant des environnements contaminés par des métaux lourds tels que le cuivre et le cadmium.

Mots clés: *Lotus ornithopodioides* L.. Métaux lourds. Phytoremédiation. Rhizobactéries, PGPR

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INTRODUCTION

Sustainable development is taking up more and more space in development policies on a global scale. It is therefore highly incompatible with an environment polluted by toxic compounds such as metallic trace elements (TM). The latter, stable and highly persistent, or even recalcitrant, compounds are considered highly contaminating compounds in the environment; can be accumulated and transferred to higher organisms in food webs leading to serious ecological and public health problems (DeForest et al., 2007; Croteau et al., 2005). Some metals are toxic even at low concentrations (Mills; et al., 1977), microorganisms happen to be the first organisms to suffer the repercussions of this toxicity (Giller et al., 1998). The presence of metallic trace elements in the edaphic environment is often reported in the literature, and it is even more surprising that no comparative study of the survival (*in situ* or *in vitro*) of bacteria sensitive or resistant to trace elements metal has not received the importance it deserves. These rhizobacteria can adapt to

unfavorable conditions as well as improving plant growth in high osmolarity environments (Rangarajan et al., 2002; Biari et al., 2008). They have developed molecular mechanisms allowing them to survive and grow with the increase in TM concentration (Tripathi; et al., 2002). According to many authors, most metal-resistant bacteria accumulate or synthesize compatible organic solutes, such as proline, glycine betaine, and betaine-glutamine. The *Bradyrhizobium* of Algerian soils, like those of other regions of the world, are more competent than the other components of the rhizobial microflora, this is in terms of growth and nodulation in extreme conditions, they confer a higher nitrogen-fixing power (Amrani et al., 2009). The aim of this research work is to isolate an endemic rhizobacteria of the genus *Bradyrhizobium* and to characterize it in order to evaluate its metallo-resistance towards copper chloride Cu(II) and cadmium chloride Cd(II) with a view to its exploitation in phytoremediation.

MATERIAL AND METHODS

1. Isolation and characterization of the bacterial strain

The endemic strain of *Bradyrhizobium* was isolated from nodulated *Lotus ornithopodioides* collected in the plots of the experimental station of the University of BLIDA 1 (Algeria). It is located between latitudes 36°29 and 36°30 and longitudes 3°35 and 3°45. The isolation of the strain of the *Bradyrhizobium* genus from the nodules was carried out according to the methodology described by Vincent (1970).

The macro and microscopic analysis of the colonies, the evaluation of their growth rates, bacterial mobility as well as the production of the enzyme Urease are carried out

according to the methodology proposed by Vincent (1970). The ability of the isolate to produce Exo polysaccharides of the Succinoglycan type (EPS I) is evaluated by the methodology of Jordan (1984). The production of indole acetic acid (IAA) is calculated following the method of Holt et al. (1994) and that of Loper et al., (1986)

2. In-vitro determination of the minimum inhibitory concentration (MIC) of trace metal elements (TM)

Two (02) milliliters of a pre-culture diluted to 1% were taken and then inoculated into two series of 250 ml capacity bottles containing 100 ml of *Tryptone Yeast Broth* (TYB) buffered medium (Beringer, 1974) having received at preliminary increasing

concentrations of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ and CdCl_2 (0 to 6 g L^{-1}) of analytical quality (Thermo Scientific Chemicals). The vials are incubated at room temperature (28°C) and in the dark for 07 days with shaking at 100 oscillations per minute. The optical density at $\lambda=600\text{nm}$ (OD_{600}) was measured in order to evaluate the minimum inhibitory concentration (MIC) of TM (Filali et al., 2000). The optical densities obtained were converted into colony forming units (CFU) based on the McFarland method proposed by Sutton (2011):

$$\text{ACC} (\times 10^8 \text{ UFC ml}^{-1}) \cong \text{DO}_{600\text{nm}} / 0,0662$$

CCA : approximative cellulaire concentration

3. Statistical analyzes

The statistical analysis of the results obtained was carried out using SPSS© software version 20.0.0 for Windows™. The experiments were repeated nine times and the analysis of normality (Shapiro - Wilk test) shows the same trends. The comparison between the means of the different treatments was established by an ANOVA followed by Tukey's post hoc test. For the comparison of the means between the concentrations according to the TM, the student test has been done. Simple linear regression analysis is performed and used for determination of MIC of Cd(II). The tests are compared to a probability of 5%.

RESULTS AND DISCUSSION

1. Characterization of isolates

The characteristics of the isolate obtained *Bradyrhizobium* sp. (*Lotus*) are summarized in Table 1. All of the nodules taken from *Lotus ornithopodoidides* L. gave bacteria which grew well on Yeast Manitol Agar (YMA) medium. The isolates presented translucent and opaque colonies, circular, convex in shape, 5 to 10 mm

in diameter. The colonies observed are completely whitish. To ensure the purity of the microorganisms present in the nodule, the result of the growth test on Glucose Peptone Agar added Bromocresol Purple (GPA+BCP) is essential, because nitrogen-fixing bacteria (NFB) have a negative reaction on this same medium. (Table 1)

Table 1: Characteristics of *Bradyrhizobium* sp. (*Lotus*)

Gram	Growth	EPS I	Mobility	Urease activity	YMA+ Congo Red	GPA+ BCP	IAA ($\mu\text{g.ml}^{-1}$)
negative	Slow	+	+	+	–	–	70.6

+ : Positive test; – : Negative test

Indeed, after incubation, growth of the isolates without change in the color of the medium is noted. A positive reaction to this test would have resulted in a mid-range color shift from deep purple to yellow. The color of the culture medium specific to the growth rate of NFB (containing Bromothymol blue) changes from green to yellow in the event of acidification of the medium. Thus, the ability to modify the pH in 24 hours allows us to distinguish fast-growing bacteria from slow-growing ones, in which the acidification of the

environment will only occur late (Vincent, 1970)

Seeding on Mannitol mobility medium shows that the isolates are mobile. Observation of the isolates in the fresh state confirms their mobility, which indicates the presence of flagella. Under optical microscope, *Bradyrhizobium* sp. (*Lotus*) appears in the form of Gram-negative pink rods.

Isolates absorb Calcofluor White (CFW) and reveal fluorescence under ultraviolet light

which indicates the production of Succinoglycan (EPS I).

The production capacity of IAA tested on *Winogradsky medium* supplemented with tryptophan indicates that *Bradyrhizobium sp.* (*Lotus*) produces $70.6 \mu\text{g mL}^{-1}$ of IAA. *Bradyrhizobia* are capable of stimulating the growth of plants, they are thus classified in PGPB (*Plant Growth-Promoting Bacteria*) organisms.

The urease activity is noted as positive during our experiment. It is expressed by the change in the color of the culture medium to indigo red

2. Metallo-resistance of *Bradyrhizobium sp. (Lotus)*

Resistance to Cd(II) and Cu(II) is defined by the minimum inhibitory concentration (MIC), which is the lowest concentration of a chemical that inhibits the visible growth of a bacteria (**Jebril *al.*, 2022**). The results obtained by measuring the average number of bacterial cells per milliliter allowed us to note that *Bradyrhizobium sp.* (*Lotus*) does not have the same growth kinetics in the presence of Cu(II) and Cd(II). The results of the experiment and their statistical treatments are reported in Table 2

Table 2: Effect of Cu(II) and Cd(II) on the growth of *Bradyrhizobium sp.* (*Lotus*)

TM concentration (g L^{-1})	<i>Bradyrhizobium sp.</i> (<i>Lotus</i>) concentration ($\times 10^8 \text{ CFU mL}^{-1}$)	
	Cu(II)	Cd(II)
0	26.71 ^{aA}	26.71 ^{aA}
1	1.02 ^{bA}	10.50 ^{cB}
1.5	0.80 ^{bA}	8.20 ^{deB}
2	0.43 ^{cA}	11.60 ^{bB}
3	0.00 ^{dA}	10.10 ^{cB}
3.5	0.00 ^{dA}	7.20 ^{eB}
4	0.00 ^{dA}	8.45 ^{dB}
4.5	0.00 ^{dA}	10.60 ^{bcB}
5	0.00 ^{dA}	8.92 ^{dB}
6	0.00 ^{dA}	8.81 ^{dB}

Means followed by the same uppercase letter in the row, and lowercase in the column, do not differ statistically from each other according to the *student t test* and the *Tukey test*, respectively, at 5% probability

2.1. Determination of the MIC of Cu(II)

At a concentration of 1 g L^{-1} of Cu (II), a significant reduction in the growth of *Bradyrhizobium sp.* (*Lotus*) ($1.02 \times 10^8 \text{ CFU mL}^{-1}$) compared to the control ($26.71 \times 10^8 \text{ CFU mL}^{-1}$) (Table 2). The reduction in growth of *Bradyrhizobium sp.* (*Lotus*) continues with the increase in the Cu(II) concentration to reach its minimum at 2 g L^{-1} ($0.43 \times 10^8 \text{ CFU mL}^{-1}$). At a copper concentration of 3 g L^{-1} there is a

cessation of bacterial development (0.00 CFU mL^{-1}). It was verified that the 2 g L^{-1} concentration of Cu (II) causes a significant absence of bacterial growth compared to the control and the other concentrations (Table 2). This result indicates that the MIC of Cu (II) is 2 g L^{-1}

2.2. Determination of the MIC of Cd(II)

The bacterial concentration in the control medium decreased significantly to reach 10.50×10^8 CFU mL⁻¹ at a concentration of 1 g L⁻¹ (Table 2). The increase in the Cd (II) concentration causes a fluctuation in bacterial growth without visible cessation, with a minimum for a concentration of 3.5 gL⁻¹ (7.20×10^8 CFU mL⁻¹), this value is significantly different from those of the other concentrations (Table 2). The resistance of *Bradyrhizobium* sp. (*Lotus*) towards Cd (II) is more pronounced compared to Cu(II), this is noted significantly in Table 2.

3. Regression analysis

3.1. Evaluation of the relevance of the regression model (Analysis of variance)

The calculation of the value of F and the associated degree of significance is recorded in the last column of Table 3. The values of F (Cd) and F (Cu) are 45.73 and 114.43 respectively; they are significant at $p < 0.0005$. This means that the chances of obtaining an F value of this size by chance are less than 0.05%. In this case, we must *reject the null hypothesis*. There is therefore a statistically significant relationship between the TM concentration and the number of bacterial cells. (Table 3)

Table 3: analysis of variance for the regression model

ANOVA ^a						
Model		Sum of squares	ddl	Average of squares	F	<i>p</i>
Cd(II)	Regression	8,166.10 ¹⁸	1	8,166.10 ¹⁸	45.73	0.000 ^b
	Residue	1,679.10 ¹⁹	94	1,786.10 ¹⁷		
	Total	2,495.10 ¹⁹	95			
Cu(II)	Regression	3,171.10 ¹⁹	1	3,171.10 ¹⁹	114.43	0.000 ^c
	Residue	8,037.10 ¹⁸	29	2,772.10 ¹⁷		
	Total	3,975.10 ¹⁹	30			

a. Dependent variable: Concentration *Bradyrhizobium* sp. (*Lotus*) (CFU mL⁻¹).

b. Predicted values: (constant), Cadmium chloride concentration (g L⁻¹).

c. Predicted values: (constant), Copper chloride concentration (g L⁻¹).

3.2. Evaluating the fit of the data to the regression model

If we square the correlation coefficient, we obtain the values R² (0.372) _{Cd} and R² (0.798) _{Cu} (Table 4). This indicates the proportion of

the variability of the dependent variable (y , number of cells) *explained* by the regression model. We can therefore say that the Cd(II) concentration can explain nearly 37% of the variation in cell growth, and that the Cu(II) concentration explains 79% (Table 4)

Table 4 : Summary of the regression model .

Summary of models ^a				
	R	R ²	Adjusted R ²	Standard error of the estimate
Cd(II)	0.572a -	0.372	0.320	4.23.10 ⁸
Cu(II)	0.893 ^b	0.798	0.791	5.26.10 ⁸

a. Predicted values: (constant), Cadmium chloride concentration (μg mL⁻¹).

b. Predicted values: (constant), Copper chloride concentration (μg mL⁻¹).

3.3. Assessment of variability explained by the regression model

Table 5 gives us the parameters of the regression model equation. The unstandardized coefficients allow us to reconstruct the equation of the regression line, which will be of the type : $y = b_0 + (b_1 \cdot x)$

The standardized coefficients column indicates the value of the correlation coefficient (Beta) which reflects a very high intensity. This value is negative, indicating that the direction of the relationship between cell growth (y) and the concentration of Cu(II) and Cd(II) (x) is inversely proportional.

According to Table 5, the regression equations are :

$$y = 1,66.10^9 - 1,66.10^5 \times x_{Cd(II)} \dots \dots \dots (2)$$

$$y = 2,26.10^9 - 1,41.10^6 \times x_{Cu(II)} \dots \dots \dots (3)$$

y : predicted value of cell growth (CFU mL⁻¹);

$x_{Cd(II)}$: concentration of Cd(II) (g L⁻¹); $x_{Cu(II)}$: Cu(II) concentration (g L⁻¹).

We thus obtain the model parameters:

$$b_{0(Cd)} = 1.66.10^9,$$

$$b_{1(Cd)} = -1.66.10^5;$$

$$b_{0(Cu)} = 2.26.10^9$$

$$b_{1(Cu)} = -1.41.10^6$$

- *In-silico* determination of the MIC of Cd(II)

Since the MIC of Cd(II) could not be observed *in vitro* , the latter is calculated using the regression equation . Based on equation (2) we obtain:

$$x_{Cd(II)} = (y - 1,66.10^9) / (-1,66.10^5) \dots \dots \dots (4)$$

By replacing “y” (number of cells) with “0” in equation (4) we obtain:

$$x_{Cd(II)} = \frac{-1,66.10^9}{-1,66.10^5} = 9,99841 \text{ g L}^{-1} \dots \dots \dots (5)$$

According to the simple linear regression model, **9.998 g L⁻¹** ($\cong 10 \text{ g L}^{-1}$) of Cd(II) is required to be able to observe a growth arrest of *Bradyrhizobium* sp. (*Lotus*). (Table 5)

Table 5: Parameters of the regression model equation

Model	Coefficients ^a			t	Sig.
	Unstandardized coefficients		Standardized coefficients		
	A	Standard error	Beta		
(Constant)	1.66.10⁹	8,534.10 ⁷		19,469	0.000
Cadmium chloride concentration (g L ⁻¹)	-1.66.10⁵	2,457.10 ⁷	-0.572	-6,763	0.000
(Constant)	2.26.10⁹	1.71.10 ⁸		13.27	0.000
Copper chloride concentration (g L ⁻¹)	-1.41.10⁶	1.31.10 ⁵	-0.893	-10.70	0.00

a. Dependent variable: Concentration *Bradyrhizobium* sp. (*Lotus*) (CFU.mL⁻¹)

DISCUSSION

The results show an acidification of the medium after 72 hours of incubation, which identifies the isolates as *Bradyrhizobium* sp. (*Lotus*) (**Mortuza et al., 2022; Vincent, 1970**) . Colonies typical of NFB absorb very rarely, or

not at all, Congo red compared to contaminating and phytopathogenic forms, or the strains occupying the nodule without fixing atmospheric nitrogen (**Jordan, 1984; Somasegaran al., 1994; Vincent, 1970**).

The α -rhizobia (including *Bradyrhizobium*) produce water-soluble exo-cellular polysaccharides, the main constituent is an acidic hetero polysaccharide (80-90%), presenting β 1-3 and/or β 1-4 bonds between these constituents parietals. Fluorescence is due to the bond between CFW and EPS I which is a type of exo polysaccharides (ESP) essential for the formation of the infectious cord at the root hairs, it plays a determining role in the construction of an effective system symbiotic nodules, particularly with legumes (**Ben Gaied et al., 2024; Jordan, 1984**). The absence of bacterial exo-polysaccharides is indicated by non-fluorescence under UV, and induces in the plant the excessive production of phenolic compounds, in particular salicylic acid and jasmonic acid. Microorganisms secrete EPS to protect themselves from the stress of different types of stress such as contamination by heavy metals, and therefore confer stress tolerance to host plants (**Upadhyay et al., 2022**).

Bacterial communities can directly affect plant growth through the production of biologically active molecules such as auxins (**Kunkel et al., 2022**). The production of this hormone is beneficial for plant growth and plays an important role in cell division, root elongation, as well as in the proliferation of root hairs (**Xiao et al., 2019**). According to **Barazani et al. (1999)**, bacteria capable of secreting a level greater than $13.5 \mu\text{g mL}^{-1}$ of indole compounds are PGPR. The work of **Ahmad, et al. (2000)** defines an IAA production of $32.8 \mu\text{g mL}^{-1}$ in the NFBs tested as high production. The rate obtained in this study ($70.6 \mu\text{g mL}^{-1}$) is therefore sufficient to stimulate plant growth and contribute to its protection.

According to the literature, microorganisms have a very active urease transforming urea into ammonia and ammonium carbonate (**SURESH et al., 2024**). According to **Györgyey et al., (2000)** if the soil contains a high concentration of nitrogen, nodulation cannot take place; the latter would act as a bacteriostatic agent and inhibit

nodulation, therefore, urease is essential to this symbiosis. NFBs contain various tolerance and/or resistance systems to TMs to maintain metal homeostasis in both bacterial and plant cells (**Jach, 2022**). Certain species of rhizobacteria resistant to TM increase the concentrations of some solutes such as thiol, Glutathione, proline, exopolysaccharides, and urease upon exposure to Cu, Ni, Zn and Cd (**Lima et al., 2006; Purchase et al., 1997; Singh et al., 2001**).

Rhizobacteria possess several protective mechanisms which increase their tolerance to very high concentrations of Cu(II) and Cd(II). These mechanisms include efflux of TMs out of the cell, extracellular sequestration, biosorption, precipitation, and bioaccumulation in cells (**Upadhyay et al., 2022**). At low concentrations, copper is recognized as an essential microelement for the growth of plants in general and microorganisms in particular. In excess, it becomes, on the contrary, toxic and growth inhibitory (**Bliefert et al., 2001**). This is confirmed by our results, where the MIC for Cu(II) against *Bradyrhizobium sp.* (*Lotus*) is 2 g L^{-1} . The result obtained indicates that the endemic strain of *Bradyrhizobium sp.* (*Lotus*) is more resistant to Cu(II) compared to several works cited in the literature. Indeed, the work of **Gharzouli et al. (2006)** show that NFB strains tested present a MIC of the order of 1.5 g L^{-1} of Cu(II).

The highest MIC of Cd(II) was noted in *Rhizobium alarii* with a value of 5 g L^{-1} (**Schue et al., 2011**), at this value the endemic strain of *Bradyrhizobium sp.* (*Lotus*) does not seem to be affected and its growth is significantly notable.

The maximum concentration (6 g L^{-1}) used during this research work does not cause a cessation of growth of *Bradyrhizobium sp.* (*Lotus*). The *in-silico* predictive analysis reveals that the MIC of Cd (II) would be of the order of $\cong 10 \text{ g mL}^{-1}$. This high tolerance of *Bradyrhizobium sp.* (*Lotus*) is explained by

the foundations of biorhizodegradation. The literature states that symbiotic microorganisms in general and a few rare genera of NFB in particular develop several detoxification systems (methylation, chelation, etc.) and transformations of pollutants of metallic origin such as Cadmium (**Jebril et al., 2022**). NBFs isolated from legumes growing in TM-contaminated soils are mainly tolerant to metal stress. The isolates are identified as *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium* and *Sinorhizobium* species tolerant to several TMEs and metalloids such as Ni, Co and Cr (**Sujkowska-Rybkowska et al., 2020; HAO et al., 2014; Dary et al., 2010**). The use of clays such as Bentonite is widely used for the adsorption of Cu(II) and Cd(II) in aquatic environments, however this approach is not compatible in the edaphic environment. The use of Bentonite is limited compared to TM. In fact, this method is much more suited to copper than to Cadmium (**Garófalo -Chaves et al., 2011**). Our results could complement the work of **Garófalo-Chaves et al., (2011)**. Indeed, *Bradyrhizobium sp.* (Lotus) belongs to the phylum *Proteobacteria* which is a major phylum of Gram-negative bacteria, the latter are generally known to be more resistant to TME, due to the composition of their cell wall. The carboxyl groups of the outer membrane (consisting of a proprietary molecule, lipopolysaccharides (LPS)) of the cell wall provide binding sites of sufficient electrical

charge to efficiently bind metal cations to the cell surface. LPS provides sufficient electrical charge, which can bind with more minerals on the cell surface (**Upadhyay et al., 2022**).

According to **Rolón-Cárdenas et al., (2022)**, auxin-producing rhizobacteria (AIA) have a beneficial effect on plants exposed to Cadmium. These bacteria decrease oxidative stress by increasing the production of antioxidant compounds and osmolytes that counteract Cd toxicity, promoting plant growth and improving the efficiency of phytoremediation. As a result, we note the appearance of a new nomenclature, *Plant Growth Stress Regulating Bacteria* (PGSRB), which defines bacteria that promote growth and regulate biotic and abiotic stress in plants (**Kunkel et al., 2022**). The role of *Bradyrhizobium sp.* (Lotus) in the avoidance of abiotic stress was studied in our subsequent work (**Bouchenak et al., 2019**). Indeed, inoculation of barley with *Bradyrhizobium sp.* (Lotus) allows the latter to accumulate a high level of osmoregulators and antioxidant molecules such as proline and soluble sugars under salt stress conditions. *Bradyrhizobium sp.* (Lotus) increases the content of chlorophyll pigments and improves the cellular integrity of barley subjected to abiotic stress, which allows us to note that this endemic strain has an effect on plants not described as hosts by the literature. (**Bouchenak et al., 2019**).

CONCLUSIONS

The conclusion drawn from the research is that the bacteria isolated from *Lotus ornithopodioides* belong to the genus *Bradyrhizobium*. These bacteria, specifically *Bradyrhizobium sp.* (Lotus), exhibit a high tolerance to copper (II) and cadmium (II), with the minimum inhibitory concentration for cadmium exceeding 6 g L⁻¹ in vitro, which is approximately 10 g L⁻¹ according to regression analysis in silicon. The endemic strain,

Bradyrhizobium sp. (Lotus), is considered a solution to the major problem of TM (essential trace metal) pollution, in addition to its ability to promote plant growth. Therefore, it is classified among the Plant Growth Stress Regulating Bacteria (PGSRB)

The research provides valuable insights into the potential of *Bradyrhizobium sp.* (Lotus) in addressing TM pollution and promoting plant

growth, particularly in the context of *Lotus ornithopodioides*.

The findings on the bacteria isolated from *Lotus ornithopodioides* have significant implications for plant growth and pollution mitigation. These bacteria could be used in

phytoremediation, biostimulation, bioaccumulation, and rhizosphere interactions, and could potentially be genetically engineered to further enhance their heavy-metal tolerance and plant growth-promoting abilities.

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