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Genetic Diversity of Nodulating Strains in the Legume *Astragalus armatus*, an Endemic Plant in Algeria

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Abstract:

Bacteria associated with leguminous species of the genus *Astragalus* (specifically *Astragalus armatus*) were isolated. The isolates were characterized through a phenotypic study, which included physiological, nutritional, and biochemical tests.A core sampling test was conducted under bacteriologically controlled conditions, following Vincent's (1970) technique. Subsequently, a total protein profile was obtained under denaturing conditions using the SDS-PAGE electrophoresis technique, as described by Laemmli(1970) . The results indicate that our isolates exhibit characteristics specific to rhizobia, in the presence of reference strains R. *leguminosarum*, *Mesorhizobium ciceri*, and *R. sullae.* In order to finalize this work, a molecular characterization was carried out. The analysis of total protein profiles under denaturing conditions using SDS-PAGE, the profiles exhibit polymorphism between the reference strains and the isolates; however, some profiles of the isolates (Ast 7, 8, 9, and 12) match that of *Mesorhizobium ciceri*. In the taxonomic aspect, particularly molecular identification, ARDRA profiles of DNA digested with the endonuclease CofI, and sequencing of the 16S rRNA gene using the Sanger technique, the NCBI blast sequence reveals the strains belonging to the γ-*Proteobacteriaceae* family and the families *Brucellaceae.* Where lead to the identification of a bacterial genus, Ochrobactrum sp., a genus recently studied and recognized as nodulating legumes.

Keywords: *Astragalus armatus* , phenotypic characterization, SDS-PAGE, molecular characterization, ARDRA, γ-*Proteobacteriaceae*, *Brucellaceae*

Introduction

Leguminous plants, the most abundant and diverse families of higher plants, are known for their ability to establish a natural mutualistic interaction with symbiotic bacteria, rhizobia, which can provide a significant source of nitrogen to natural and agricultural ecosystems. Leguminous family includes annual herbaceous plants as well as woody plants and can be found in all regions of the world. They hold significant economic importance, some species play a critical ecological role, particularly in stabilizing and fertilizing soil (acacias) or serving as excellent green manure. The ability of most legume species to develop symbiotis, can be a major source of nitrogen , potentially reducing the need for chemical nitrogen fertilizers. This interaction is perhaps the most well-known ecological characteristic of the legume family, as it requires the presence of compatible, competitive, and efficient microbial partners (de Lajudie et al., 2019; Rcha and al., 2020). Despite the abundance and diversity of legumes, relatively few studies have focused on the symbiotic relationship between legumes and rhizobia. Therefore, our study aimed to address this gap by characterizing the diversity and characteristics of rhizobia associated with *Astragalus armatus*, an endemic legume plant in Algeria, has received limited attention in the literature despite its ecological and agricultural potential. To better understand the diversity and characteristics of rhizobia associated with *this plant*, we conducted phenotypic and genotypic study on bacterial isolates from the nodules

Astragalus is a potential candidate for revegetation programs in arid and semi-arid regions in Algeria (Mahdjoub and al., 2019). However, little is known about the rhizobia associated with this plant species. Thus our aim is to isolate and characterize the rhizobia associated with *A. armatus* and to assess their potential for nitrogen fixation. Our goal is to understand the genetic diversity of nodulating strains associated with this plant, through a series of phenotypic and biochemical tests, and total protein profiling using SDS-PAGE. Our approach is based on the well-established protocols developed by Vincent (1970) and Laemmli (1970), as well as the more recent studies on the ecology and diversity of rhizobia (de Lajudie and al., 2019; Rcha and al., 2020). Strains isolated from the roots of the legume *Astragalus armatus* are subjected to phylogenetic characterization, in particular an ARDRA profile, by digestion of the DNA by an endonuclease, and sequencing of the 16S rDNA according to the Sanger protocol. This last part is carried out with the kind collaboration of the Department of Biotechnology of the University of Padua (Italy). Through this study, we hope to contribute to the growing body of knowledge on legume-rhizobium symbiosis and the potential for the use of these symbiotic associations in sustainable agriculture and soil restoratio

Materials and Methods

Sampling zone and plant material

The collection of nodules is carried out from the roots of the *Astragalus armatus*, endemic fodder legume described by Quezel and Santa (1962) as a 20-50 cm shrub with more or less diffuse stems with leaf rachis becoming indurated and transformed into very strong thorns.

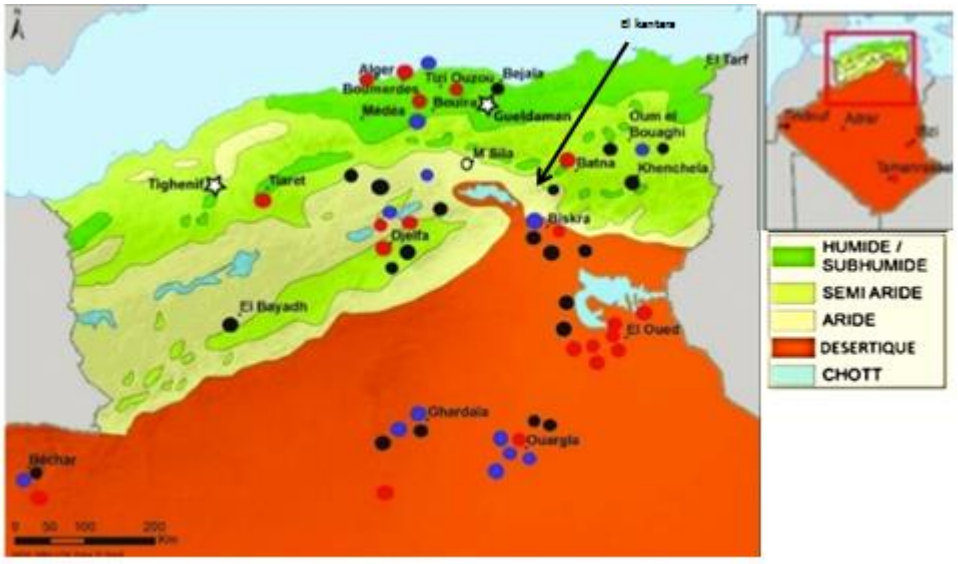


Figure 1: Geographic location of the sampled site in south-eastern Algerie: ElKantara, located in the southwest of the Aurès, 52 km north of Biskra and 62 km southwest of Batna. The natural site of El Kantara and the Roman heritage have been classified and protected since 1923.

Isolation of bacteria from nodules

Sterile nodules are individually crushed. The procedure is performed under total aseptic conditions (laminar flow hood, flamed forceps). nodule juice is spread onto Petri dish containing Yeast-Mannitol-Agar (YMA medium), supplemented with Congo red. The seeding is done using the four-quadrant streaking technique to obtain isolated colonies that are easy to characterize. The same nodules are seeded on a Petri dish containing Glucose Peptone Agar supplemented with bromocresol purple (GPA) and Agar with Bromothymol Blue (BTP), media successively to identify contaminant colonies and determine the growth rate. This step is followed by a microscopic and morphological study of the isolates (Gram staining and colony appearance on YMA according to Vincent, 1970, Jordan, 1984 and Somasegaran and Hoben, 1994.The plates are then incubated for three days at 28°C. This method is used for the isolation and cultivation of bacteria from sterile nodules, commonly employed in the study of nitrogen-fixing bacteria such as rhizobia.

Distinctive test between Rhizobium and Agrobacterium

A series of tests differentiating between Rhizobium and Agrobacterium species are applied to our isolates: calcium glycerophosphate precipitation (Hofer, 1941), 3-ketolactose test (Bernarts and De Ley, 1963), and growth on Litmus milk (Vincent, 1970).

Biochimistry caracteristics of strains

Search for associated enzymes

As the bacteria are associated with the plant nodules through a process of infection we investigate enzymes associated with infection and specific to rhizobia: urease (Somasegaran and Hoben, 1994), pectinase (polygalacturonase), cellulose: endoglucanase (Teather and Wood., 1982), and nitrate reductase (Behringer, 1974)

Determination of protein profil

The ability of the isolates to form nodules under aseptic conditions and in the presence of germinated legume seeds is assessed according to Vincent (1970) using Leonard jars. A total protein profile is performed using the Laemmli (1970) method on polyacrylamide gel under denaturing conditions (SDS-PAGE) at pH 6,8.

Abiotic test

Several environmental conditions limit the growth and activity of nitrogen-fixing plant pH, temperature, and salt. to assess the bacteria's tolerance to these stress factors, our isolates and reference strains were cultured in their presence . Using the medium described by Gloux and Le Rudulier (1989), with NaCl added at the following concentrations: 20 mM, 40 mM, 100 mM, 200 mM, 400 mM,600mM, 800mM, 1 M, 1.5 M, and 2 M.. Moreover, to estimate the optimal and maximum growth temperatures, in another hand to evaluate the stress pH, the strains were cultured in liquid BIII medium and incubated at different temperatures: 4°C, 28°C, 30°C, 37°C, 40°C, 42° and 45°C and at different pH values: 5, 5.8, 6.8 (control), 7.8, 8.8, and 9.8. Growth is evaluated in each tube by measuring the optical density at 600 nm after 36 hours and 48 hours of incubation

Nodulation test: proceeding

To identify isolates, we studied their capacity and aptitude to form nodules with the host plant, *Astragalus armatus,* under bacteriologically controlled conditions. l (Graham and al., 1991). Nodulation tests should be conducted in traditional Leonard jars (Vincent, 1970) or appropriate tubes (Beck and al., 1993), we have used the two methods.

Sterilization of seeds and germination

The seeds are immersed in 95% ethanol for 10 seconds to remove surface contaminants. They are then treated with concentrated sulfuric acid solution for 10 minutes to eliminate potentially present pathogenic microorganisms on the seeds and facilitating effective scarification of the seeds accelerates their germination. The seeds are thoroughly rinsed with sterile distilled water; a total of 10 rinses are performed to remove any residual ethanol and sulfuric acid. Finally, the seeds are left submerged in the final rinse for 5 to 6 hours to rehydrate after sterilization

Sterile seeds are placed for germination on culture plates containing semi-solid TYA medium (7g/l agar) . TYA is a nutrient medium used for bacterial growth and seed germination. The culture plates are wrapped in aluminum foil to maintain darkness, as some seeds require dark conditions for efficient germination.

Inoculation

After germination, the seeds are planted in a sand-vermiculite mixture (top part of the jar) at a rate of 2 to 3 seeds per jar, and then immediately inoculated with 2 ml of an exponentially growing bacterial suspension (OD600 ≈ 1). Two jars are left uninoculated and will serve as controls. Finally, the jars are placed in a growth chamber at room temperature with controlled lighting for eight weeks (Somasegaran, 1994).

Molecular characterization

The molecular characterization of the isolated strains is carried out with the assistance and kindness of Professor A. Squartini of the Agrarian Biotechnology Laboratory, University of Padua (Italy), and is carried out by the ARDRA technique according to the experimental protocol cited by Squartini and al, 2002

DNA extraction

The extraction protocol is essentially the same regardless of the nature of the sample. Cells are lysed by placing a loopful of the isolation colony in 50 µl of lysis buffer (0.25% SDS, 0.05M NaOH) in an Eppendorf tube, shake for 1 minute on a vortex then heat to 95° C for 15 minutes. The lysate is centrifuged for 15 minutes at 12,000 rpm (Eppendorf 5415D type centrifuge), 10 µl of the supernatant is taken and added to 90 µl of sterile water. The rest of the lysates are stored at -20°C before PCR.

Amplified Ribosomal DNA Restriction Analysis (ARDRA)

This analysis was conducted on 16S rDNA by the of Restriction Fragment Length Polymorphism.One µl of diluted lysate containing all the DNA of the bacterial isolates is processed by a BioRad I-Cycler PCR type device using the two universal bacterial 16S rDNA target primers, 63F (5'CAGGCCTAACACATGCAAGTC) (Marchesi and al. , 1998) and 1389R (5'ACGGGCGGTGTGTACAAG) (Osborn and al., 2000) at 1µM, are placed in a reaction volume of 25µl, using the following program: initial denaturation at 95°C for 2 minutes; 35 cycles at 95°C for 30 sec, 55°C for 1 minute, 72°C for 4 minutes and a final elongation at 72°C for 10 minutes. 12µl of the amplificat in 25µl of the reaction volume, are digested overnight at 37°C with 1.5µl of the restriction enzyme CfoI (Pharmacia, Upsala, Sweden) and 1.5µl of 10x reaction buffer. The fragments obtained after digestion are separated according to their size by electrophoresis on 2% agarose gel during three hours at 110 V. The colored gel is visualized using a UV transilluminator and photographed using a high resolution Kodak DC290 digital camera.

Sequencing using the Sanger method

Dideoxyribonucleotides are labeled using fluorescent dyes, with a different color (Big Dye, PerkinElmer/Applied Biosystems, Foster City Calif. U.S.A). The four reactions can thus take place in the same test tube. Sequencing is performed on an ABI prism Applied Biosystems 3730xl automated sequential DNA counter. Chromatograms are analyzed by the Chromas 2.23 DNASTAR program (Technelysium Pty Ltd, Tewantin Australia).The 16S rDNA fragments sequenced from the strains studied are compared to all the sequences already stored in the databases (Altschul and al., 1990) NCBI website (http://www.ncbi.nlm.nih .gov /).

Results and discussion

The isolates originating from the homogenized nodules of the legume *Astragalus armatus* underwent a series of subculturing on YMA medium to ensure the purity of these cultures. The bacterial isolation yielded twelve isolates as listed in the table below (Table 1). All isolates are Gram negative.

Table1: strains extracted from *Astragalus armatus* (endemic plant)

Strains	Host Plant	Geographic Origin

Ast 1, Ast 2, Ast3, Ast4, Ast5, Ast6, Ast7, Ast8, Ast9, Ast10, Ast11, Ast12	*Astragalus armatus*	El kantara (region of Biskra)

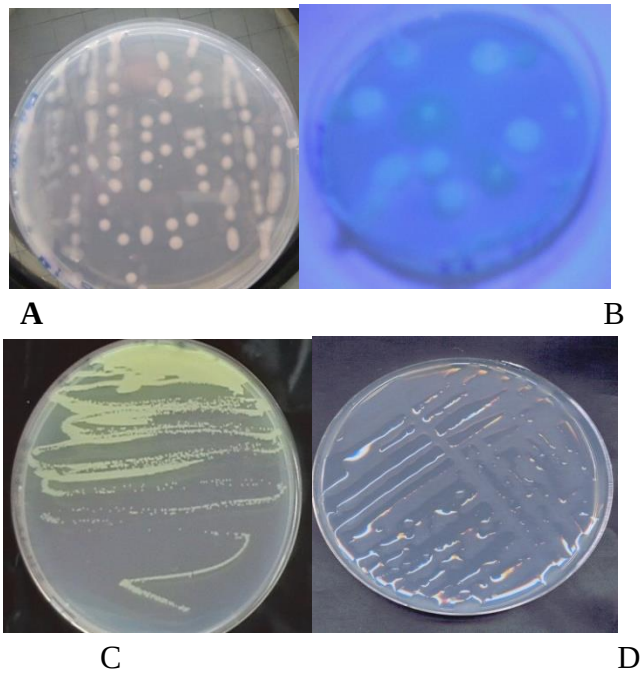
Based on the results obtained, the isolated strains from the legume *Astragalus armatus* exhibit the same morphological characteristics like reference strains (Table 2). Indeed, all strains grow within 24 hours on YMA or YMA supplemented with Bromothymol Blue, and do not cause acidification on Glucose-Peptone-Agar medium in the presence of Bromocresol Purple

Table 2: Reference strains

Code	Strains	Host plant	Geographic Origin	Source
A6	*Rhizobium sullae A6*	*Hedysarum coronarium*	Constantine, Algeria	A. Benguedouar – Constantine
T	*R. leguminosarum bv. Trifolii*		B.J.Rolfe, Canberra	B.J.Rolfe, Canberra
V	*R. leguminosarum bv. Viciae*		Rothamstead Collection	Rothamstead Collection
CIII	*Mesorhizobium cicero*	*Cicer arietinum*	Constantine, Algeria	S. Dekkiche – Constantine

Distinguishing tests.

The microscopic and morphological features, distinguishing tests, reactions to Congo Red and Calcofluor, and the growth rate suggest that our isolates exhibit characteristics consistent with the Rhizobium genus (Vincent, 1970, 1982; Jordan, 1984; Chen and al., 1991; Ge Hong Wei and al., 2006).(figure 1).



A B

C D

Figure 1: Morphological features A: Absorption of Congo Red (0.0025%), B: Absorption of Calcofluor, C: Growth Rate on BTP, D: YMA without Dye

Biochimistry caracteristics of strains

Search for associated enzymes

We observed that both the isolates and reference strains produce polygalacturonase, cellulase (endoglucanase), nitrate reductase, and urease, enzymes associated with the root infection and nodule-forming mechanisms (Table 3).

Growth of isolates and control strains on KNO3-based medium, followed by addition of nitrate reductase reagents, demonstrates the presence of the enzyme. (Lindström and Lehtomaki, 1988; Struffi and al., 1998). Nitrates inhibit the activity of nitrogenase in legume nodules (Lucinski and al., 2002).All strains produce urease on specific medium supplemented with urea. Its presence is indicated by alkalization of the medium (production of NH3).

Table3: Strains 'enzymes activities

Strains / **Enzyme activity**	***Ast1***	***Ast2***	***Ast3***	***Ast4***	***Ast5***	***Ast6***	***Ast7***	***Ast8***	***Ast9***	***Ast10***
Cellulose activity	++	++	++	++	++	++	++	++	++	++
Urease activity	++	++	++	++	++	++	++	++	++	++
Pectinase activity	++	++	++	++	++	++	++	++	++	++

++: Best production of enzymes

Total protein profile

The profile showed polymorphism among the isolates. This polymorphism revealed three distinct clusters: a group1: Ast.1, 3, 4 isolates, group2 Ast.5, 6, 7, 8, and the third one: Ast.9, 10, 11, 12.

It is noteworthy that the profiles of the isolates Ast 7, 8, 9, and Ast 12, 13 are almost identical to that of *R. ciceri* (10) (figure 3). Finally, the bacteria isolated from the nodules of *Astragalus armatus* show profiles that are quite distinct from those of *R. leguminosarum bv. trifolii, R. leguminosarum bv, viciae*, and *R. sullae* species. SDS-PAGE is an initial approach for identifying legume-nodulating bacteria, comparing SDS-PAGE banding profiles to group strains into homogeneous or heterogeneous clusters (Dekkak 2018., 2019. Almi and al 2020).

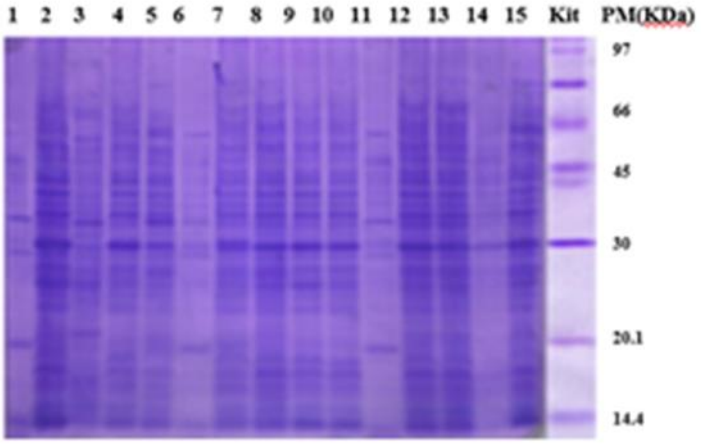


Figure 3 : Total protein profil; 1: *R.L.t* ; 2 : *Ast 1* ; 3:*Ast 3*; 4:*Ast 4*; 5:*R.L.v* ; 6:*Ast 5* ; 7:*Ast 6* ; 8:*Ast 7*; *9:Ast 8* ; 10:*M.R.c* ;11:*Ast 9*;12:*Ast 10* ; 13:*Ast 11* ;14:*Ast 12* ;15:*R.sullae*

Determination of molecular weights of the analyzed proteins:

According to Coyne and al., 2002, we calculate the frontal ratio or relative mobility of each polypeptide band from each strain using the following formula (Table 3)

Rf = distance traveled by the protein / distance traveled by the marker

Frontal ratio	Molecular weight (Da)	Log $_{10}$ (MW)
Rf1= 0.3/6.8= 0.04	**97 000**	**4.98**
Rf2= 0.8/6.8= 0.11	**66 000**	**4.81**
Rf3= 1.5/6.8= 0.22	**45 000**	**4.65**
Rf4= 3.3/6.8= 0.48	**30 000**	**4.47**
Rf5= 4.8/6.8= 0.70	**20 100**	**4.30**
Rf6= 6.1/6.8= 0.89	**14 400**	**4.15**

We observe the presence of a specific polypeptide band with a molecular weight of 30 kDa, which is consistently present in most isolates of the reference strain *Mesorhizobium ciceri*. This polypeptide band could represent a particular protein expressed characteristically by this bacterial species.

Abiotic tests

Most isolates tolerate NaCl concentrations up to 400 mM, the isolates exhibit optimal growth conditions (DO= 0,09) similar to reference strains. However, strain *Ast 11* tolerates a significantly higher salinity concentration up to 800mM (DO=0,02). Beyond 1000 mM, no strain grows (DO= 0). The results indicate a tolerance threshold of 400 mM. It is important to note that tolerance to a specific NaCl concentration does not necessarily indicate good growth of the strains. Azib (2020) demonstrated a negative effect of increasing NaCl concentrations on the growth of bacteria nodulating alfalfa. The tolerance is often attributed to the presence of osmoprotective molecules in bacterial cells (Nour and al., 1995).

All isolates, along with the reference strains, showed growth across a temperature range from 4°C to 42°C. However, at 45°C, no growth was observed. Similarly, certain alfalfa nodulating isolates were able to grow up to 42°C (Kajic and al., 2019). Our isolates show tolerance to low temperature (4°C). In contrary, Wei andt al (2008) showed that the mesorhizibium, cannot tolerate a temperature of 4°C. Notably *Lathyrus* and *Phaseolus*, which can tolerate temperatures up to 45°C (Mohammed and al., 2020; Rocha and al., 2020). Additionally, The study conducted by Tewari and al. in 2020 characterized rhizobia capable of nodulating pigeon pea and surviving temperatures as high as 45°C. This highlights an intriguing adaptation of these microorganisms to hot environmental conditions.

Our isolates were unable to grow at acidic pH levels (pH 5). they were able to withstand alkaline pH levels (pH 9.8).Our results are actually similar to those of Dekak et al., 2018. On the other hand, Mellal's work (2019) detected that strains isolated from the legume *Lupinus angustifolius* were more sensitive to alkalinity than to acidity.

We know that the nitrogen supply provided by symbiosis is limited by the autoregulation of nodule formation, and the host plant receives the physiological minimum of nitrogen. This minimum can sustain the host plant's survival but may not be sufficient to support high-yielding legume crops. Most importantly, we understand that symbiosis is a short-term phenomenon, and its duration may not be adequate to sustain a plant throughout its entire life until seed maturation. Shanker and al., (2020) have thoroughly discussed the impact of abiotic stress factors not only on the growth and survival of rhizobial bacteria but also on the mechanisms by which these bacteria adapt to arid and semi-arid conditions by regulating the expression of genes involved in tolerance to stressful environmental factor. Fedorova and al., (2024), have shown that symbiosis efficiency is restricted by environmental conditions.

Symbiotic performance between isolates and host plant

Growth measurement

To assess the growth of strains in nodulating legume seeds, survival curve determination allowed us to select isolates in their exponential phase at a wavelength of 540 nm, which could reach up to 2.0 (after 18 hours of incubation) (Beringer, 1974).

Seeds germination

The seeds germinate approximately 5 to 6 days after treatment (10 min in sulfuric acid). In the other hand, Kheloufi and al (2019) showed that for the Arid Steppe ecotype of Aïn Naga region of Biskra, the most effective treatment was immersion in sulfuric acid for 60 minutes. In contrast, for the Condorcet Mountain ecotype, the effective treatment duration was shorter, only 30 minutes. This type of treatment is likely being used to achieve a specific experimental outcome, such as seed scarification (breaking or softening the seed coat to promote germination) or tissue preparation for genetic or physiological studies.

Nodutation test

The nodulation test under aseptic conditions, involving the connection between the isolates and germinated seeds of the plant, was conducted in a controlled room for ten weeks. The bacteria isolated from the nodules of *Astragalus armatus* exhibit characteristics common to the Rhizobium genus, including morphology, fast growth rate, and reactions to Congo red and Calcofluor, according to Jordan

(1984) The results showed that only two isolates, Ast 11 and Ast 8, were able to form very few nodules (maximum of two) on the roots of the leguminous plant.(figure number 5).

Figure 5: Roots of nodulated *A. armatus* by the isolate *Ast 11*

Enzyme assays and total protein profiling also suggest similarity to Mesorhizobium ciceri. However, recent studies on the *Astragalus* species highlight the prevalence of Mesorhizobium huakuii (Chen, W.X and coll., 1991).

Profile ARDRA and rDNA 16S Séquençing

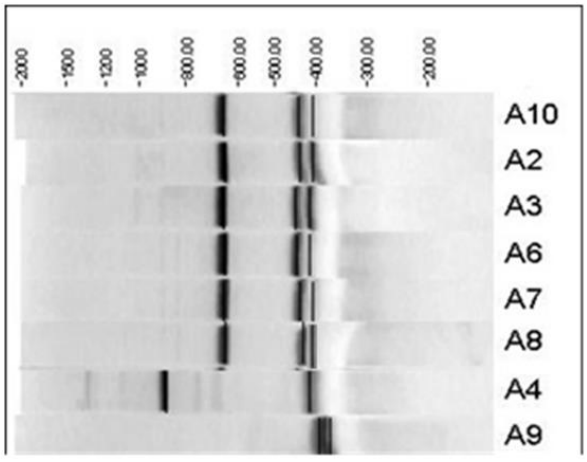


Figure 6: ARDRA profile of isolates after DNA digestion by CfoI endonuclease

The ARDRA profile on Agarose gel of the isolates reveals three clusters with three different profiles (fig number 6):

- A common profile bringing together the Ast 10, Ast 2, Ast 3, Ast 6, Ast7, Ast8 strains
- A unique profile for the Ast 9 strain,
- A unique profile for the Ast 4 strain.

The sequencing of the 16S rDNA, according to the Sanger technique, of the same isolates, referring to the Blast, NCBI sequence, gives us the following results (Table 4).

Table 4 :Strains Blast

Isolats	Souche	% similarity
Ast 9	*Ochrobactrum*	100%
Ast 10,Ast 2, Ast 3, Ast 6, Ast 7, Ast 8	*Enterobacter sp.*	100%
Ast 4	*Bacterium HPC438*	82%

Ast9 is the only one isolat that belongs to *Ochrobactrum*, all the other are rhizobiaceae forming two groups; *Enterobacter* and *Bacterium*.

Ezzakkioui 2015 also found molecular biodiversity of isolates from partial sequencing of the 16S ribosomal gene related to the legume of the genus *Hedysarum*, including differential similarities to the species *Rhizobium sullae IS123T* of the family *Rhizobiaceae* of the class *α-proteobacteria*; and the genera *Stenotrophomonas, Serratia, Massilia, Acinetobacter, Pseudomonas* and *Achromobacter* of the *β* and *ɤ-proteobacteria* classes.

El Batanony and al., 2020 chowed that *Astragalus* and other plants grown-wild at different geographical locations in Egypt also contained bacterial species of the genera *Achromobacter*, *Ancylobacter*, *Bacillus*, *Cronobacter*, *Curtobacterium*, *Flavobacterium*, *Paenibacillus* and *Pseudomonas*.

Msaddak and al., 2023 have shown that the majority of rhizobia nodulating the legume genus *Lupinus* belong to different genera, notably the genus *Bradyrhizobium*, which includes strains phylogenetically related to various slow-growing species, as well as fast-growing bacteria belonging to the genera *Microvirga*, *Ochrobactrum*, *Devosia*, *Phyllobacterium*, *Agrobacterium*, *Rhizobium*, and *Neorhizobium*.the one who walks our results. This suggests that these fast-growing lupin-nodulating bacteria probably acquired their symbiotic genes from rhizobial genera other than Bradyrhizobium through horizontal gene transfer.

In their work, Shamseldin and Velázquez (2020) observed that Phaseolus vulgaris L., a leguminous plant, is capable of nodulating with at least 27 species of Rhizobium from four different genera of bacteria. These species were isolated from nodules of P. vulgaris and other leguminous plants, hence the origin of symbiovars. Therefore, it is considered a host with high promiscuity.

CONCLUSION

The current study focus on the isolation and characterization of bacteria associated with the roots of *Astragalus armatus*, an endemic leguminous plant in Algeria. In this study, based on various phenotypic and biochemical tests, the isolates were identified as members of the *Rhizobium* genus

The study of abiotic factors (NaCl, pH, and temperature) highlights the following finding:

Both the strains isolated from the roots of the legume *Astragalus armatus* and the reference strains tolerate relatively high concentrations of

The growth of the isolates is observed across a range of pH levels, from acidic to alkaline. This indicates adaptability to varying soil pH conditions.

Given that the roots are sampled from an arid soil the isolates grow well at temperatures around 40-42°C. This suggests they are adapted to the high temperatures typical of arid environments.

The strains isolated from *Astragalus armatus* roots demonstrate resilience to high NaCl concentrations, versatility across pH ranges, and adaptation to elevated temperatures typical of semi-arid climates. These characteristics underscore their potential suitability for symbiotic relationships with the legume under such environmental conditions. In fine: The rhizosphere promotes plant survival under adverse conditions

The isolates exhibited polymorphism and formed three distinct clusters, with some isolates exhibiting similarity to *Mesorhizobium ciceri*.. This study provides important insights into the genetic diversity of nodulating strains in *Astragalus armatus* and highlights the potential of this plant for use a nitrogen source in agricultural ecosystems.

In the taxonomic aspect, notably the molecular identification, the ARDRA profiles of the DNA digested against the CofI endonuclease, and the sequencing of the 16S rDNA according to the Sanger technique, lead to a bacterial genus, *Ochrobactrum sp*., recently genus studied and listed as Bacteria Nodulating Legumes (Zurdo_Pineiro and al., 2007).

Perspectives projects: Using an appropriate technique, it is possible to correctly identify novel bacterial species with superior nitrogen fixing abilities. These strains would be vital in developing inoculation programs and boost legume production

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