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# Phenotypic and symbiotic characterization of bacteria nodulating *Genista saharae* in the arid region of Algeria

Caracterización fenotípica y simbiótica de las bacterias que nodulan *Genista saharae* en la región árida de Argelia

Caracterização fenotípica e simbiôntica de bactérias noduladoras de *Genista saharae* na região árida da Argélia

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## Abstract

Twenty bacterial strains had been isolated from root nodules of *Genista saharae* that grew wild in Biskra and El Oued city (Northeastern Algerian Sahara). This study focused on obtaining isolates of legume nodule bacteria (LNB) from the plant *G. saharae* and evaluated their effectiveness in forming a symbiotic relationship with the legume species *Vigna unguiculata* through cross-inoculation. Additionally, the study aimed to identify the successful cross-inoculation group of LNB strains based on their phenotypic characteristics. The growth capacity of isolates under varying salinity conditions [NaCl] and pH levels was investigated using a spectrophotometer (96-microplate reader). The API 20NE and API 20E systems were used to identify the biochemical characteristics of the isolates. In addition, the rhizospheric soil samples from the two study sites were analyzed using standard analytical techniques of soil. All isolates established effective symbioses with *Vigna unguiculata*, were Gram-negative rods and were fast-growing. The optimal growth temperature was between 28 °C and 37 °C; some isolates were thermophiles and specifically withstood extreme heat between 45-50 °C. Furthermore, they demonstrated a wide tolerance range to pH (5–10) with salt tolerance ranging from 100 mM to 500 mM. Biochemical results revealed that the isolates assimilated various sources of carbon and nitrogen and displayed numerous enzyme activities. Physicochemical analysis revealed that all the soils were deficient in nutrients and had an alkaline pH. This study enabled us to identify the effective stress-tolerant strains, which could be used in the future to inoculate plants for environmental applications.

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## Resumen

Se aislaron veinte cepas bacterianas de los nódulos radiculares de *Genista saharae* que crecían de forma silvestre en Biskra y El Oued, en el noreste del Sahara argelino. Este estudio se centró en obtener aislados de bacterias nodulares de leguminosas (BNL) de *G. saharae* y evaluar su eficacia en formar una simbiosis con *Vigna unguiculata* mediante inoculación cruzada. Además, se identificaron las cepas BNL exitosas según sus características fenotípicas. La capacidad de crecimiento de los aislados bajo diferentes condiciones de salinidad [NaCl] y niveles de pH se investigó utilizando un espectrofotómetro (lector de microplacas de 96 pocillos). Los sistemas API 20NE y API 20E se emplearon para identificar las características bioquímicas de los aislados. Se analizaron muestras de suelo rizosférico de ambos sitios utilizando técnicas analíticas estándar. Todos los aislados establecieron simbiosis efectivas con *Vigna unguiculata*, eran bacilos Gram-negativos y de rápido crecimiento. La temperatura óptima de crecimiento fue entre 28 °C y 37 °C; algunos aislados resistieron calor extremo (45-50 °C). Además, mostraron amplia tolerancia al pH (5-10) y a la sal (100 mM a 500 mM). Los resultados bioquímicos indicaron que los aislados asimilaron diversas fuentes de carbono y nitrógeno, exhibiendo numerosas actividades enzimáticas. El análisis fisicoquímico reveló suelos deficientes en nutrientes y con pH alcalino. Este estudio identificó cepas tolerantes al estrés, útiles para futuras aplicaciones ambientales.


**Palabras clave**: rizobios, caracterización morfofisiológica y bioquímica.


## Resumo

Foram isoladas vinte estirpes bacterianas de nódulos radiculares de *Genista saharae* que cresciam espontaneamente em Biskra e El Oued, no nordeste do Saara argelino. Este estudo focou na obtenção de isolados de bactérias nodulares de leguminosas (BNL) de *G. saharae* e avaliou sua eficácia na formação de uma relação simbiótica com *Vigna unguiculata* por meio de inoculação cruzada. Além disso, identificaram-se as estirpes BNL de sucesso com base em suas características fenotípicas. A capacidade de crescimento dos isolados sob diferentes condições de salinidade [NaCl] e níveis de pH foi investigada com um espectrofotômetro (leitor de microplacas de 96 poços). Os sistemas API 20NE e API 20E foram usados para identificar as características bioquímicas dos isolados. Adicionalmente, as amostras de solo rizosférico dos dois locais de estudo foram analisadas com técnicas analíticas padrão. Todos os isolados estabeleceram simbioses eficazes com *Vigna unguiculata*, eram bastonetes Gram-negativos e de rápido crescimento. A temperatura ótima de crescimento foi entre 28 °C e 37 °C; alguns isolados eram termófilos e resistiram ao calor extremo (45-50 °C). Além disso, mostraram ampla tolerância ao pH (5-10) e à salinidade (100 mM a 500 mM). Os resultados bioquímicos revelaram que os isolados assimilaram diversas fontes de carbono e nitrogênio e exibiram várias atividades enzimáticas. A análise físico-química mostrou que todos os solos eram pobres em nutrientes e tinham pH alcalino. Este estudo identificou estirpes tolerantes ao estresse, úteis para futuras aplicações ambientais.


**Palavras-chave:** rizóbios, caracterização morfofisiológica e bioquímica.

## Introduction

The Genisteae is one of the largest tribes within the legume family (Fabaceae), comprising 618 species across 25 genera (Cardoso *et al.*, 2013). *Genista saharae* Coss & Dur is a spontaneous shrub legume thriving in the Sahara. However, as a legume, it is able to fix atmospheric nitrogen ($N_2$) via symbiotic association with bacteria termed rhizobia in its root nodules, contributing to soil fertility (Mahdhi *et al.*, 2007). The use of these bacteria as bio-inoculants increases the availability of nutrient elements in soil, helps to minimize the chemical fertilizer application, reduces environmental pollution, and promotes sustainable agriculture. Several studies aim to identify new symbiotic strains resistant to extreme environmental conditions, such as the research conducted by Zakhia *et al.* (2004) finds that two strains isolated from *Genista microcephala* Coss. & Durieu, grown in an infra-arid region of Tunisia, are identified as *Rhizobium*. Mahdhi *et al.* (2007) reveal that novel isolates nodulating *G. saharae* originating from the infra-arid region of Tunisia are linked to various species of rhizobia belonging to the genera *Ensifer* (75 %) and *Rhizobium* (10 %). Chaïch *et al.* (2017) show that the majority of isolates nodulating *G. saharae*, a plant thriving in the hyper-arid zone of the northern Algerian Sahara, are attributed to the genus *Ensifer meliloti* (formerly known as *Sinorhizobium*). Furthermore, they identify *Neorhizobium,* comprising three distinct species: *N. alkalisoli*, *N. galegae*, and *N. huautlense* along with *Mesorhizobium*, represented by the species *M. camelthorni*. In contrast to fast-growing strains, the genus *Bradyrhizobium* (slow-growing strains) appears to be a predominant group that nodulates the majority of *Genisteae* species in Northeastern Algeria (Boulila *et al.*, 2009; Ahnia *et al.*, 2018; Boudehouche *et al.*, 2020). However, previous studies (González-Andrés *et al.*, 1998) find that other *Genista* species such as *G. tinctoria* growing in Poland (16 strains), Ukraine (17 strains), and England (10 strains), as well as *G. monspessulana* and *G. linifolia* in Spain, are nodulated by only *Bradyrhizobium* spp. However, there is no information available about the microsymbionts associated with the legume *G. saharae* growing wild in two distinct geographic locations in Biskra and El Oued city (Northeastern Algerian Sahara). The current study, therefore, aims to: (i) investigate the diversity of symbiotic bacteria associated with *G. saharae* by phenotypic methods; including morphological, physiological, and biochemical characterization, (ii) evaluate their symbiotic effectiveness in order to select the efficient stress-tolerant strains.

## Materials and methods

### Study sites

In this study, soil samples, root nodules were collected from *G. saharae* plants growing at two different sites. The first site is situated in the Bouchagroune region (34.80°N / 5.73°E) (Biskra city). The second site, located at Oued El alanda (Erg Chegamet) (33° 14’ N / 6° 14’ E) (El Oued city). El Oued and Biskra are located in the northeastern part of the Algerian Sahara.

### Physico-chemical analysis of rhizospheric soils

### Soil sampling

Soil samples were collected from the rhizospheres of *G. saharae* and were analyzed for organic carbon (OC) content, total nitrogen (N), available P, pH, texture, electric conductivity (EC) according to (Aubert, 1978; Mathieu *et al.*, 2003).

**Nodule collection and Isolation of bacteria**

Nodules were collected from *G. saharae* roots at depths ranging from 30 cm to 1 meter. They were carefully detached and stored in tubes containing silica gel (Somasegaran and Hoben, 1985). The bacterial isolation from nodules was conducted in accordance with the protocol followed by Vincent (1970), Somasegaran and Hoben (1985). Nodules were rehydrated overnight at 4 °C by immersion in distilled water and then surface-sterilized for 30 seconds in 96 % ethanol and for 5 minutes in a 3.5 % (v/v) sodium hypochlorite solution. They were then washed five to ten times using sterile distilled water. Each nodule was crushed in an Eppendorf tube, and the resultant suspension was streaked on Yeast Extract Mannitol Agar (YMA) media plates. The plates were then incubated for 24 to 48 hours at 28 °C. Individual colonies were checked for purity by repeated streaking on fresh YMA medium and by microscopic examination of cellular morphology and Gram-stain reaction. Purified strains were conserved at 4 °C on YMA slopes containing 0.3 % $CaCO_3$ for short-term use and were cryogenically preserved at -20 °C on Yeast Extract Mannitol Broth (YMB) with 20 % glycerol (v/v) for long-term storage.

**Plant Nodulation and symbiotic effectiveness test**

The isolates were tested for cross-nodulation on *Vigna unguiculata* (L.) Walp plants, which is characterized by its high promiscuity (Pongslip, 2012) and rapid growth. To perform this test, we utilized the Leonardo's jar technique (Vincent, 1970). Plastic water bottles were cut in half, cleaned with detergent, disinfected with ethanol, and connected with sterilized medical compresses. The bottom part was filled with a nutrient solution ($CaCl_2$ (0.10 $g.L^{-1}$), $MgSO_4 \cdot 7H_2O$ (0.12 $g.L^{-1}$), $KH_2PO_4$ (0.10 $g.L^{-1}$), $Na_2HPO_4 \cdot 2H_2O$ (0.15 $g.L^{-1}$), Ferric citrate (0.005 $g.L^{-1}$), Trace elements (1 mL). Trace elements: ($H_3BO_4$ (2.86 $g.L^{-1}$), $MnSO_4 \cdot 4H_2O$ (2.03 $g.L^{-1}$), $ZnSO_4 \cdot 7H_2O$ (0.22 $g.L^{-1}$), $CuSO_4 \cdot 5H_2O$ (0.08 $g.L^{-1}$), $Na_2MoO_4 \cdot 2H_2O$ (0.14 $g.L^{-1}$)). The pH was adjusted to 6.8, and sterilization was performed at 120 °C for 20 minutes, and the upper part was filled with sterilized sand. The medical compress cord connecting the two parts allowed for the moistening of the sand with the nutrient solution. The seed surface was sterilized in 96 % ethanol for 5 to 10 seconds, left for 2 hours in sterile distilled water (SDW) and germinated on sterile cotton soaked in sterile distilled water, in darkness at 25 °C. The seeds underwent a germination period of 24 hours at 23 °C. The germinated seeds were then planted at a density of three seedlings per plastic pot, each containing 300 g of sterilized sand (120 °C for 20 minutes) and inoculated with 2 mL of an early stationary-phase bacterial culture cultivated at 28 °C in YMB broth. The plants were grown in a growth chamber with a 16/8 h light/dark photoperiod at 25 °C/18 °C day/night. Three replicates were performed per isolate with uninoculated negative controls (N-free). Six weeks post-inoculation, the plantlets were harvested and examined for the presence of root nodules.

**Phenotypic characterization**

One hundred eighty (180) µL of YMB medium containing the corresponding NaCl concentrations in millimolar (mM) (100, 200, 300, 400, 500, and 1.7 mM which served as control) and 180 µL of YMB containing pH variations (pH 4, pH 5, pH 6, pH 7, pH 8, pH9, pH 10 and pH 6.8 which served as control) were distributed into the wells of a microplate. [NaCl] concentration 1.7 mM (0,1 $g.L^{-1}$) and pH 6,8 were involved in the formulation of the medium YMB broth medium. Then, 20 µL of inoculum ($1.10^9$ $CFU.mL^{-1}$) for each bacterial isolate were added. Each test was conducted with three repetitions, and wells containing uninoculated YMB served as negative controls. The microplates were incubated at 28 °C with orbital shaking (150 rpm) for 48 to 72 hours. The growth of the bacterial cultures was monitored by measuring the optical density (OD) at 600 nm using a microplate spectrophotometer (96-microplate reader Thermoscientific Multiskan Sky) (Lindström and Lehtomäki, 1988). To assess the maximum and optimal growth temperatures, the isolates were inoculated onto YMA medium, with three repetitions for the test, and incubated at various temperatures: 26 °C, 32 °C, 37 °C, 44 °C, 50 °C and 28 °C which served as control chosen by Zakhia *et al*. (2004). Readings were taken after 24 to 48 hours of incubation. To identify the biochemical characteristics of the isolates, API 20NE and API 20E strips were used. The strips were inoculated following the instructions provided by BioMérieux (BIOMERIEUX REF 20050 Api 20NE) and (BIOMERIEUX REF 20100/20160 Api 20E).

**Statistical analysis**

The influence of [NaCl] concentration and pH levels on the growth of isolates was investigated using a one-way Analysis of Variance (ANOVA) in SPSS, version 2020, followed by the Tukey post hoc test. The phenotypic character results were coded numerically in a binary table where "1" represented a positive result and "0" a negative result, using PAST software (version 4.03, 2020, folk.uio.no/ohammer/past). Hierarchical clusters were generated in the UPGMA algorithm using 1000 bootstrapped Jaccard similarity (Sj).

## Results and discussion

**Physicochemical characteristics of rhizosphere soils**

The rhizosphere soil varied in its physicochemical properties. Bouchagroune soil had a sandy texture with a high sand content 78.79 % while the clay and silt content were 0 %, and it was characterized by alkaline pH (8.20). Oued El Alanda soil also had a sandy loam texture. The clay and silt content were 0 % and 19.06 %, respectively and it was characterized by alkaline pH (8.03). The texture of the two different sites indicated low water retention. The electrical conductivity (EC) values of Bouchagroune and Oued El Alanda sites were 0.2 $ms.cm^{-1}$ and 0.3 $ms.cm^{-1}$, respectively. The soils were classified as non-saline according to the agronomic scale (Scianna *et al.*, 2007). The nitrogen and available phosphorus levels were: Site 01; (N) 0.056 %, (P) 51.20 ppm and Site 02; (N) 0 %, (P) 52.08 ppm. The organic matter percentages from the two studied sites were both less than 1 %. So they characterized by low content of organic matter. Chemical analysis revealed that all the soils were poor in nutrients, specifically available (N) and (P). Plant Pi levels played a crucial role in shaping the plant-associated microbiota and regulating the endosymbiosis with soil microbes (Míguez-Montero *et al.*, 2020). When legumes encountered low Pi levels, they exhibited a decrease in nodule formation and a reduction in the nitrogen-fixing capacity within the developed nodules (Ma and Chen, 2021).

**Rhizobial isolation and symbiotic effectiveness**

A collection of twenty (20) legume nodule bacteria (LNB) was selected from the root nodules of *G. saharae* (table 1). All isolates grew rapidly on YMA medium. Colonies appeared mucoid, white, and creamy in color. They were Gram-negative rods. All strains were able to induce nodulation on the roots of *Vigna unguiculata*. In contrast, the non-inoculated control plants did not produce any nodules, confirming the infectivity of the tested strains. All strains induced the formation of ineffective white nodules, meaning they did not fix nitrogen (Ahnia *et al.*, 2018), as well as effective red nodules,

leghemoglobin accumulation, a hemoprotein exclusive to nitrogen-fixing nodules (Downie, 2005). The development of symbiotic nodules on legume roots was controlled by a host genetic program (Tsyganov and Tsyganova, 2020). Several hosts, such as *V. unguiculata* (cowpea), were known to be highly promiscuous (Pongslip, 2012). The absence of nodules depended on several extrinsic and intrinsic factors.

**Phenotypic characterization and numerical taxonomy**

Phenotypically, all isolates were able to grow at pH levels between 5 and 10 (figure 1). However, pH 4 had a negative impact on bacterial growth (Tukey HSD, $P < 0.05$), as seen in figures 1 and 1.2. All isolates were able to grow at pH 6.8 with significant differences observed between isolates (Tukey HSD, $P < 0.05$), where Bg38 showed the best growth at pH 6.8, while OS07 exhibited the lowest growth at this pH, as seen in figure 1.1. The optimum pH for growth was between 6.8 and 8 (figure 1). Most of the tested isolates tolerated NaCl concentrations from 100 mM to 500 mM (Tukey HSD, $P<0.05$), as shown in table 1. According to the Tukey HSD test, the best bacterial growth was recorded at 100 mM and 200 mM and the lowest growth was recorded at 500 mM (figure 2). The Tukey model showed that the increase in NaCl concentration from 100 mM to 500 mM resulted in a significant decrease ($P<0.05$) in the growth rate of all tested isolates (figure 2). All the selected isolates were able to grow at 1.7 mM (Tukey HSD, $P < 0.05$). Bg38 showed the best growth at 1.7 mM, while OS07 exhibited the lowest growth at this concentration (figure 2.1). Concerning the isolates' response to different temperatures. The results obtained showed that the majority of strains from two different sites were able to grow at 26 °C, 28 °C, 32°C and 37 °C. Approximately 25 % (two strains from Oued El Alanda site and four strains from Bouchagroune site) were able to withstand temperatures ranging from 44 °C to 50 °C (table 1). Mahdhi *et al.* (2007) demonstrated that the majority of the isolates of *G. saharae* from Tunisian soil were capable of growth within a pH range of 6 to 12. However, none of the isolates were able to grow at pH 4, while Dekak *et al.* (2018) demonstrated that the isolates of *Genista microcephala* and *Argyrolobium uniflorum* (*Genisteae*) were able to grow in acidic and alkaline pH values ranging from pH 3.5 to pH 10. Extreme pH levels affected nodulation by reducing infection by rhizobia. Highly acidic soils and highly alkaline soils affected the survival and growth of both partners, thus reducing nitrogen fixation (Bordeleau and Prévost, 1994). Succinoglycan might have played a role in adaptation to low pH (Hawkins *et al.*, 2017) and contributed to survival in nodules (Maillet *et al.*, 2020). Most of the isolated strains of *Genista saharae* in the Algerian Sahara tolerated up to 4 % NaCl and grew at 45 °C (Chaïch *et al.*, 2017).

Mahdhi *et al.* (2007) confirmed that all of the isolates were resistant to high temperature and most of them continued to grow at 40 °C. In addition, the majority of the isolates were able to grow in high NaCl concentrations (3 %). This may have been a specific adaptation to the high soil temperatures and salinity in arid regions. These results confirmed those obtained by Mahdhi *et al.* (2007) and Chaïch *et al.* (2017).

**Table 1. Phenotypic characteristics of bacterial strains isolates the root nodules of *Genista saharae*.**

| Phenotypic characteristics | | Strains isolated from *Genista saharae* | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Bouchagroune site | | | | | | | | | | Oued El alanda site | | | | | | | | | |
| | | Bg57 | Bg51 | Bg40 | Bg53 | Bg05 | Bg17 | Bg28 | Bg39 | Bg20 | Bg50 | Bg79 | Bg34 | Bg80 | Bg38 | Bg42 | Bg60 | Os02 | Os05 | Os07 | Os10 |
| Nodule number | | 06 | 11 | 05 | 24 | 23 | 08 | 14 | 11 | 16 | 12 | 17 | 13 | 06 | 04 | 07 | 08 | 18 | 10 | 02 | 02 |
| Temperature | 26 °C | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 28 °C © | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 32 °C | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + |
| | 37 °C | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 44 °C | - | - | - | - | + | - | - | NR | + | - | + | + | - | - | - | - | - | - | + | + |
| | 50 °C | - | - | - | - | + | - | - | - | + | - | + | + | - | - | - | - | - | - | + | + |
| pH | 4 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 5 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 6.8 © | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 8 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 9 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 10 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| NaCl mM | 1.7 © | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 100 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 200 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 300 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 400 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 500 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

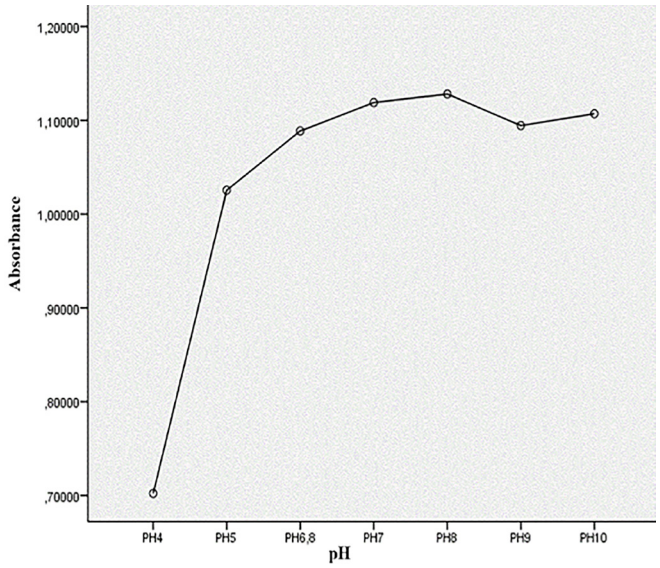


**Figure 1. pH effect on the growth of *Genista saharae* microsymbiont isolates (Tukey HSD, P < 0.05).**

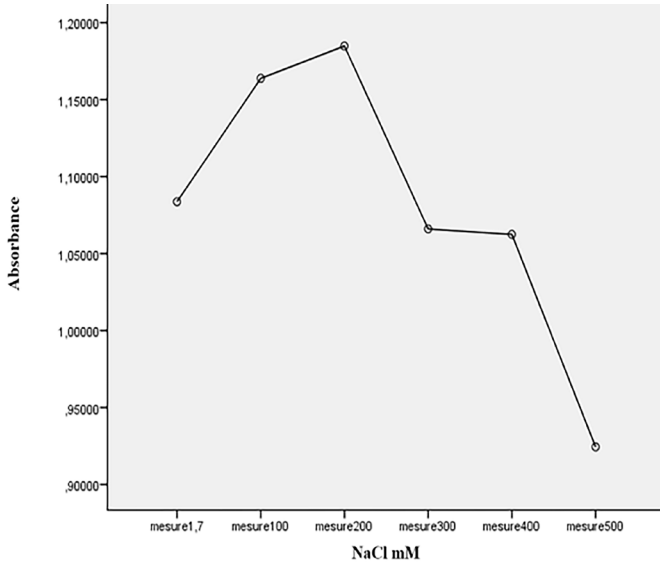


**Figure 2. [NaCl] effect on the growth of *Genista saharae* microsymbiont isolates (Tukey HSD, P < 0.05).**

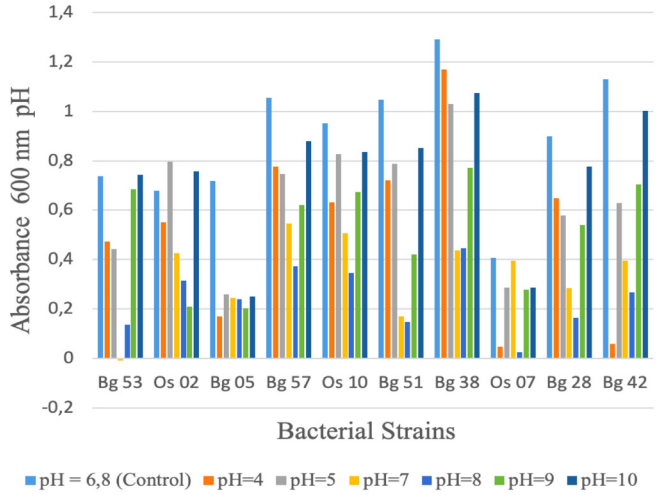


**Figure 1.1. pH effect on the growth of *Genista saharae* microsymbiont isolates.**

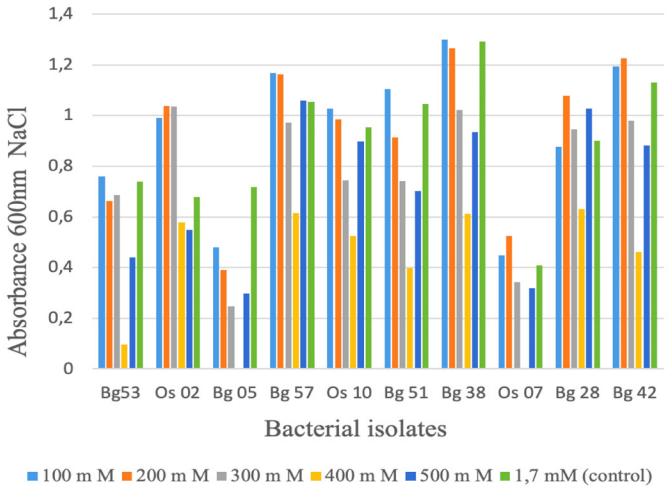


**Figure 2.1. NaCl effect on the growth of *Genista saharae* microsymbiont isolates.**

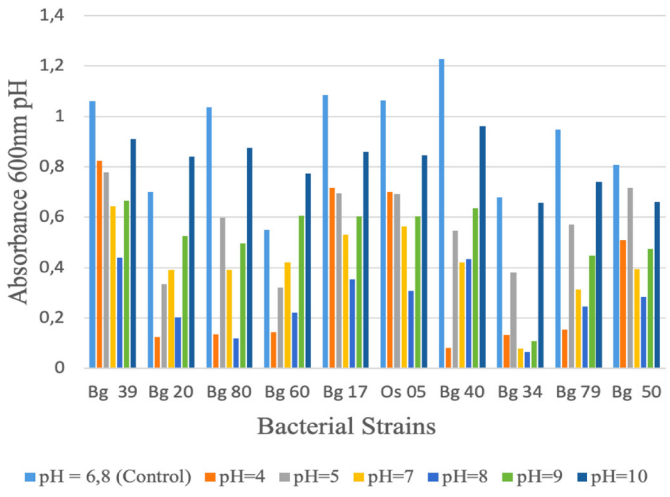


**Figure 1.2. pH effect on the growth of *Genista saharae* microsymbiont isolates.**

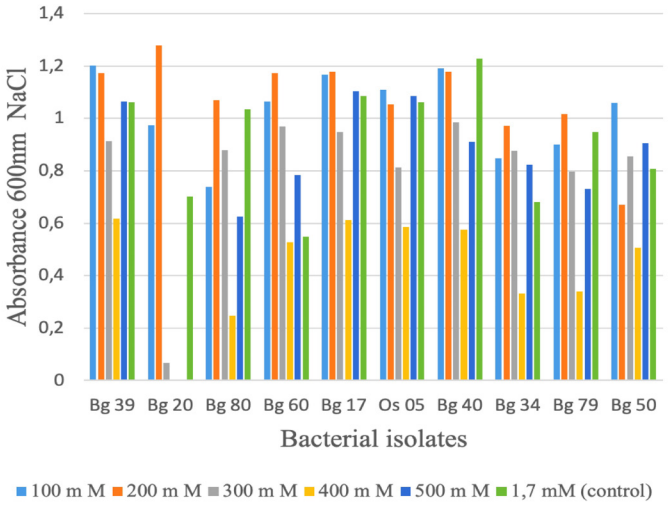


**Figure 2.2. NaCl effect on the growth of *Genista saharae* microsymbiont isolates.**

The ability to survive in such extreme environments was conferred by adaptation mechanisms that enabled them to withstand salt stress (Miller and Wood, 1996). Some studies reported that different species of *Rhizobium* presented adaptation mechanisms related to the production of Heat Shock Proteins (HSPs) at temperatures beyond their normal growth range, as noted by Michiels *et al.* (1994). This confirmed the resistance of some strains in our study to high temperatures, ranging from 44 °C to 50 °C. The isolates demonstrated variability in assimilation of carbon and utilization of nitrogen sources, as well as enzymatic activity. Numerical taxonomic analysis based on the 51 variable features (tables 1 and 2) revealed two distinct classes (I and II) at a Jaccard similarity coefficient level of 0.84, as shown in (figure 3), because bacterial isolates were obtained from two different regions, Biskra and El Oued. The two different classes generated five different clusters. The first cluster included the strains (Bg28, Bg 57, Bg 51, Bg 17, Bg 39, and Bg 79) from *Genista saharea* (Bouchagroune). For example, the subgroup (Bg17 and Bg39) showed 100 % similarity, used all carbon sources tested except for inositol, adipic acid, and capric acid. They assimilated and fermented glucose as a source of energy. They used ornithine and arginine as nitrogen sources but did not use tryptophan and lysine because they did not have tryptophan desaminase and lysine decarboxylase. They

**Table 2. Biochemical characteristics of bacterial strains isolates the root nodules of *Genista saharae*.**

| Biochemical characteristics | | Strains isolated from *Genista saharae* | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Bouchagroune site | | | | | | | | | | | Oued El alanda site | | | | | | | | |
| | | Bg57 | Bg51 | Bg40 | Bg53 | Bg05 | Bg17 | Bg28 | Bg39 | Bg20 | Bg50 | Bg79 | Bg34 | Bg80 | Bg38 | Bg42 | Bg60 | Os02 | Os05 | Os07 | Os10 |
| API Test | NO3 | + | + | - | + | + | + | + | + | + | - | + | - | - | + | - | - | - | + | - | + |
| | TRP | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | TDA | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | IND | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | ADH | + | + | - | - | - | + | + | + | - | - | - | - | - | + | - | - | - | + | - | + |
| | LDC | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | ODC | + | + | - | - | - | + | + | + | - | - | + | - | - | + | - | - | - | - | - | - |
| | URE | + | - | - | - | - | + | + | + | - | - | - | - | - | - | - | - | - | + | - | + |
| | ESC | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | GEL | + | + | + | + | + | - | + | - | - | + | - | + | + | - | + | + | + | + | + | - |
| | H2S | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | PNPG | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + |
| | ONPG | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + |
| | VP | - | - | - | - | - | - | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| | $GLU_f$ | + | + | - | - | - | + | + | + | + | - | + | - | - | + | - | - | - | + | - | + |
| | $GLU_a$ | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | ARA | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | MNE | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | MAN | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | NAG | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + |
| | MAL | + | + | - | + | - | + | + | + | + | + | + | - | + | - | - | - | + | + | - | + |
| | GNT | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | CAP | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - |
| | ADI | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - |
| | MLT | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | CIT | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | PAC | + | + | - | - | - | + | + | + | + | - | + | - | - | - | - | - | - | + | - | + |
| | INO | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - |
| | SOR | + | + | - | - | - | + | + | + | - | - | + | - | - | + | - | - | - | - | - | - |
| | RHA | + | + | - | - | - | + | + | + | - | - | + | - | - | + | - | - | - | - | - | - |
| | SAC | + | + | - | - | - | + | + | + | - | - | + | - | - | + | - | - | - | - | - | - |
| | MEL | + | + | - | - | - | + | + | + | - | - | + | - | - | + | - | - | - | - | - | - |
| | AMY | + | + | - | - | - | + | + | + | - | - | + | - | - | + | - | + | - | - | - | - |

**(+): positive, (-): negative, NR: growth but not resistant, ©: control**

**ONPG** (β-galactosidase), **ADH** (Arginine Dihydrolase), **LDC** (Lysine Decarboxylase), **ODC** (Ornithine Decarboxylase), **CIT** (Citrate utilization), **H2S** (Hydrogen sulfide production), **URE** (Urease), **TDA** (Tryptophan Deaminase), **IND** (Indole production), **VP** (Voges-Proskauer - Acetoin production), **GEL** (Gelatinase), **GLU** (Glucose fermentation/oxidation), **MAN** (Mannitol fermentation/oxidation), **INO** (Inositol fermentation/oxidation), **SOR** (Sorbitol fermentation/oxidation), **RHA** (Rhamnose fermentation/oxidation), **SAC** (Sucrose fermentation/oxidation), **MEL** (Melibiose fermentation/oxidation), **AMY** (Amygdalin fermentation/oxidation), **ARA** (Arabinose fermentation/oxidation).**NO3** (Nitrate reductase), **TRP** (Indol production), $GLU_a$ (Glucose assimilation), $GLU_f$ (Glucose fermentation), **ARA** (Arabinose assimilation), **MNE** (Mannose assimilation), **MAN** (Mannitol assimilation), **NAG** (N-acetyl-glucosamine assimilation), **MAL** (Maltose assimilation), **GNT** (Gluconate assimilation), **CAP** (Caprique acid assimilation), **ADI** (Adipate assimilation), **MLT** (Malate assimilation), **PAC** (Phenylacetic acid assimilation), **PNPG** ( (β-galactosidase para-Nitrophenyl-β-D-galactopyranoside), **ESC** (Esculin hydrolysis).

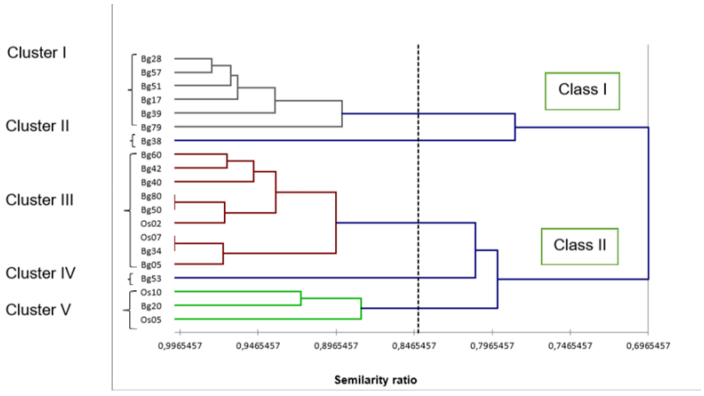


**Figure 3. UPGMA dendrogram showing the similarities based on phenotypic tests between *Genista saharae* strains isolates.**

had the nitrate reductase enzyme, which reduced nitrate to nitrite. The strains were considered as aero-anaerobic lithotrophs. They produced esculin, urease, β-galactosidase, and did not have gelatinase and sulfate reductase, so they could not produce hydrogen sulfide, because they were aerobic bacteria. Both strains could not withstand 44 °C. Cluster II included Bg38. The most particular characteristic of strain Bg38 was that it could not grow at 32 °C and its optimal growth occurred at a salinity of 1.7 mM (Tukey HSD, $P<0.05$) (figure 2.1). It did not produce indole, lysine decarboxylase, urease, gelatinase, and β-galactosidase. It could not use N-acetyl-glucosamine, maltose, adipic acid, phenylacetic acid, and inositol as sources of carbon. The third cluster regrouped (Bg60, Bg42, Bg40, Bg80, Bg50, Os02, Os07, Bg34, Bg05). The strain of Os02 from *G. saharea* (Oued El Alanda) formed a subgroup with Bg50 and Bg80. It differed from them in its inability to use adipic acid as a source of energy. Cluster IV included one isolate, Bg53, from *G. saharea* (Bouchagroune). It was characterized by its inability to grow beyond 40 °C, non-production of indole, ornithine decarboxylase, lysine decarboxylase, and urease. It produced nitrate reductase, gelatinase, esculin, and β-galactosidase. It assimilated glucose, arabinose, mannitol, N-acetyl-glucosamine, maltose, potassium gluconate, adipic acid, and citrate. Cluster V consisted of three strains (Os10, Bg20, Os05): these strains used ornithine as a nitrogen source and could not use sorbitol, rhamnose, sucrose, melibiose, and amygdalin as sources of energy. They could withstand up to 50 °C, except for Os05, for which the maximum resistance was 40 °C. An important biochemical diversity was observed. The isolates demonstrated variability in enzymatic activity; some isolates were chemo-heterotrophs, capable of fermentation and exhibit facultative aero-anaerobic respiration. This extraordinary metabolic flexibility allowed the bacteria nodulating legumes to possess great adaptive capacities in response to extreme environmental conditions.

## Conclusion

In general, phenotypic studies show a large physiological and biochemical diversity of selected isolates, exhibiting the basic characteristics of rhizobia. In addition, the isolates show variable tolerance to different stress factors (temperature, pH, salinity). The present study enables us to identify the most effective strains nodulating *Genista saharae* growing in arid soils of the Northeastern Algerian Sahara, which we can use in the future to inoculate plants for environmental applications such as the restoration of degraded and poor soils. However, the work needs to be completed by studying genotypic biodiversity to identify new effective genospecies.

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