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ISOLATION AND CHARACTERIZATION OF MESORHIZOBIUM STRAINS FROM WILD AND CULTIVATED CHICKPEA NODULES IN THE KURDISTAN-IRAQ

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ABSTRACT

The study was conducted to investigate symbiosis and node-forming ability of *Mesorhizobium* bacteria in chickpea (*Cicer arietinum* L.) growth under challenging environmental conditions. Nodules were collected from wild and cultivated chickpeas in the Kurdistan Region. Endophytic bacteria associated with their roots were isolated and characterized. All the 30 isolates, were found to be gram-negative with smooth, circular colonies. Most isolates (80%) were mucoid, while 20% were not and produced green colonies on bromothymol blue. Half of the isolates 50%) grew best in alkaline pH (6.6-10.5), 26.6% thrived in acidic pH (3.5-5.5), and 20% preferred neutral pH. Regarding salt tolerance, 63% of the isolates grew well in 0-4% NaCl, while 6.67% tolerated up to 6% NaCl. In terms of temperature, 56.67% grew better in the range of 15-35°C, while 36.67% thrived in 25-40°C. Some isolates had slow growth in alkaline conditions, producing blue colonies, while fast-growing isolates in acidic conditions yielded yellow colonies. This research provides insights into the adaptability of these bacteria under harsh conditions of high pH, salinity, and extreme temperatures. The outcomes offering a pathway to improving chickpea resilience and productivity in marginal lands of the Kurdistan Region and similar sites worldwide, through utilizing indigenous candidate rhizobia for adverse environments.

Keywords: *Mesorhizobium*, Chickpea nodule, symbiosis, gram-negative bacteria, salinity tolerance, indigenous rhizobia.

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INTRODUCTION

Agriculture plays a critical role in sustaining human life by providing essential food and nutrition. Increasing demand on food due to population rise and climate change is becoming a big threat to crop production. Especially in arid regions of Asia (Habib-ur-Rahman et al, 2022). Kurdistan- Iraq is located in the core of the global climate change as a semi-arid region with harsh climate encountering hot summer and cold winter weather (Forti et al, 2023). Legumes are among various promised crops with high protein source for human consumption and animal feeding (Singh et al, 2022; Khdir et al, 2023) Chickpea is found as one of the effective pulse legumes to reduce the impacts of climate change effects particularly in semi-arid areas (Nyaombo, 2022, Sabri Dizayee, 2023). It belongs to the monogeneric tribe Cicereae, subfamily Ppilionoideae (Fabodea) of Fabaceae family, having nine annuals and 34 perennial species in the genus *Cicer* (Singh, 2015). Cultivated chickpea (*Cicer arietinum* L.) is an annual herbaceous belongs has been cultivated for thousands of the years and is a staple food in many parts of the world (Mabrouk, 2018,) and is an important cool season pulse crop (Jadhav, 2021). Chickpea (*Cicer arietinum*) domestication was identified

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to be domesticated about 10,000 years BP in the Fertile Crescent (Igolkina, 2023). Then the domesticated chickpeas were further spread from the Fertile Crescent toward other sites around the world (Varshney, 2021). Study of soil microbiomes revolutionized understanding of ecosystem, integral component of sustainable agriculture (Bier, 2024). Endophytic bacteria as a soil microbiome in root zone have a direct impact on nutrition provision and plant health (Abdullaeva et al, 2022; Adeleke et al, 2021; Teja et al, 2022). Such bacteria have a crucial role in many biochemical processes which take place in the soil and highly beneficial for plant growth, plant health and soil properties (Yadav, 2020). Environmental factors such as soil acidity, salinity, nutrient deficiency and extreme temperature have a negative effect on the legume nodulation and even limiting the vigor of the legume host (Swarnalakshmi et al, 2020). It is revealed that naturally occurring rhizobial populations in a particular geographical region have more nodulation competency than inoculant strains (Rai, 2012). There are many studies on the improvement of yield, disease resistance and nutritional values of chickpea while less have been realized on addressing the genetic diversity of chickpea rhizobia (Gebremedhin et al, 2018), Information on endophytic bacteria is still with a big gap and only a few plants (1-2% of all known plants) have been studied to determine their endophyte composition (Strobel, 2018). This creates a great opportunity to discover new and useful endophytic microorganisms in plant diversity for different ecosystems (Emitaro et al, 2014), especially for the indigenous rhizobia under local stressed environment. Hence, characterizing indigenous chickpea rhizobia populations could be beneficial to improve the symbiotic association of chickpeas and their rhizobia (Zhang et al, 2024). Generally, unavailable atmospheric nitrogen converts to available form of ammonia through different pathways. Plant utilizes available nitrogen to synthesize different proteins, vitamins, and other nitrogen containing compounds (Kumar et al, 2016, Nath Bhowmik et al, 2018). Rhizobia is one of the symbiotic nitrogen fixers which is controlled by nitrogenase enzymes, which is the cost effective and environment friendly procedure in which rhizobia interacting with leguminous plants and fix aerobic nitrogen into soil. The presence of rhizobia increases plant productivity without any harm to human health and environments also assist to reducing reliance on chemical fertilizers (Hamza, T.A. and Alebejo, 2017). The objectives of the current study were to: isolate and characterize Mesorhizobium strains collected from nodules of wild and cultivated chickpeas in the Kurdistan-Iraq and assess their growth and survival performance under different environmental stressors, to identifying bacterial strains that enhance chickpea resilience and productivity in adverse environments. Also to investigate the symbiotic relationship between Mesorhizobium bacteria and chickpea (*Cicer arietinum* L.), with a focus on the bacteria's ability to form nodules and improve plant growth under challenging environmental conditions such as high pH, salinity, and temperature extremes.

MATERIALS AND METHODS

Sample collection: The present study was conducted at College of Agricultural Engineering Sciences, Department of Biotechnology and Crop Science, University of Sulaimani, Kurdistan-Iraq. Chickpea roots with attached nodules and surrounding rhizospheric soil were collected from six distinct sites at the Kurdistan-Iraq, for three wild and three cultivars in the spring of 2021. Five random plants were selected in each site at flowering stage for nodule collection. Thirty bacteria endophytes were isolated from collected root nodules of chickpea from six distinct regions. Wild chickpeas (*Cicer oxydon*) were collected in Sartaki-bamo, Penjween and Dere sites. Cultivated chickpeas (*Cicer arietinum*) were collected from Qasre, Harir and Xarpane. Wild chickpea grows on mountain areas were sampled with different elevations of 1488m, 1395m and 1742m for Penjween, Dere and Sartaki-bamo, respectively. The GPS coordination and soil characterization for the collection sites were stated in Table 1.

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Table 1. GPS coordination, Climatic condition, and some soil characteristics of chickpea (*Cicer* spp.) at different collection sites

Parameters	Penjween	Dere	Harir	Xarpane	Sartaki-bamo	Qasre
Latitude	35° 35.42 N	35° 56.93 N	36° 32. 16 N	35° 13.47 N	34◦ 58 N	36° 34. 59 N
Longitude	45° 58.13 E	45° 34.06 E	44° 19.04 E	46° 02.23 E	45◦ 5.0 E	44°49. 05 E
Elevation (masl)	1488	1395	611.2	787.4	1742	1178
Rainfall (mm Y^{-1})	888	907	456	410	464	847.5
Soil texture	Silty loam	Loam	Clay loam	Silty loam	Silty clay	Clay
Organic matter (%)	1.26	1.79	1.66	1.110	1.19	1.79
pH	7.08	7.10	7.98	7.90	7.50	7.95
EC (dSm^{-1})	0.30	0.28	0.26	0.37	0.25	0.25
Available P ($\mu g\ g^{-1}$)	3.048	15.145	11.416	2.899	5.625	8.543
Available K ($\mu g\ g^{-1}$)	0.106	0.186	0.10	0.115	0.066	0.075
N%	0.22	0.28	0.13	0.25	0.29	0.29

Surface sterilization of root nodules and Isolation of endophytic bacteria

Surface sterilization, and bacteria culturing were performed according to Vincent and Humphrey (Vincent and Humphrey, 1970). Sterilized forceps were used to crush sterilized nodules in 20µL sterile distilled water. The resulted suspension was spread in a Petri dish and further streaking was conducted on YEM plates and incubated at 28±2°C for 7 days.

Morpho-Cultural and physiological characterization of the Isolates

The rhizobial isolates were tested to tolerate different levels of pH on YMA medium 3.5, 4.5, 5.5, 6.5, 6.8, 7.5, 8.5, 9.5 and 10.5 pH). HCl of 10 N and 1 N of NaOH were used to adjust the pH of YMA medium. After culturing bacterial isolates on YMA medias, they were incubated at 28°C for seven days and the influence of pH on bacterial growth was recorded every 24 h (L'taief et al, 2007). Figure (1) showed the impact of pH on bacteria strains.

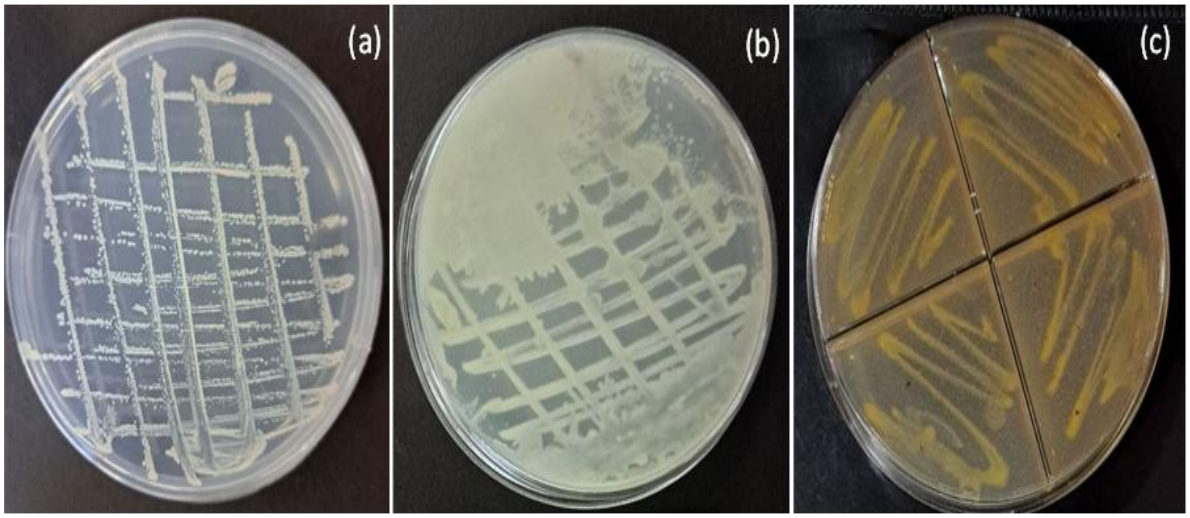

Figure 1. Clarify the effect of different pH on bacterial strains; (a) the effect of pH 3.5 on QC95, (b) effect of pH 8.5 on WS1, and (c) is the effect of alkaline pH 10.5 on WD47

Bromothymol blue test was performed to assess the acid/alkali production of each chickpea rhizobia isolates. Based on the color change of the medium, isolates were categorized as green to yellow, fast growers/acid producer or green to blue slow growers/alkali producer, respectively (Sharma, et al, 2010) as indicated in Figure 2.

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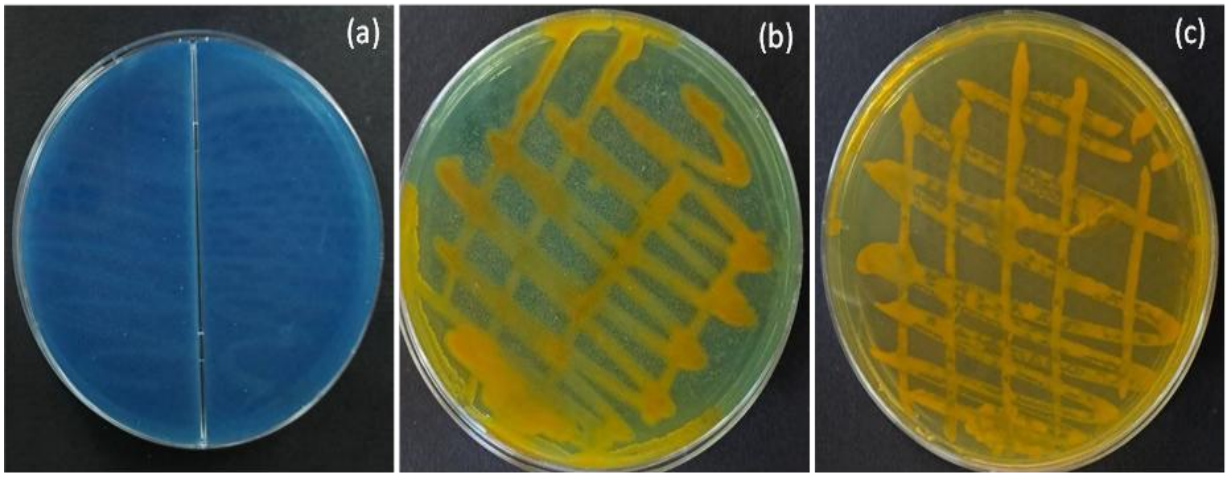

Figure 2. Show different color of bacteria when grow on yeast mannitol agar contain bromothymol blue (pH indicator), (a) producing alkaline blue colony WS29, (b) is neutral green colony WS46, (c) showing acidic fast yellow colony WD45

The impact of temperature on growth of each chickpea rhizobia was also assessed using YMA media. Each isolate was inoculated on YMA media and incubated at various temperatures including 15, 20, 25, 28, 30, 35 and 40 °C, the data documented every 24 h. (Maataln et al, 2002). The tolerance of chickpea rhizobia isolates to sodium chloride (NaCl) was evaluated using YMA medium enriched with variable NaCl concentrations (0, 0.1, 0.5, 1, 2, 4 and 6%) according to Kenasa, Jida (Kenasa et al,2014), Figure 3. Gram staining is the fundamental technique used in microbiology to distinguish gram negative and positive based on structural difference in their cell walls use Hucker method.

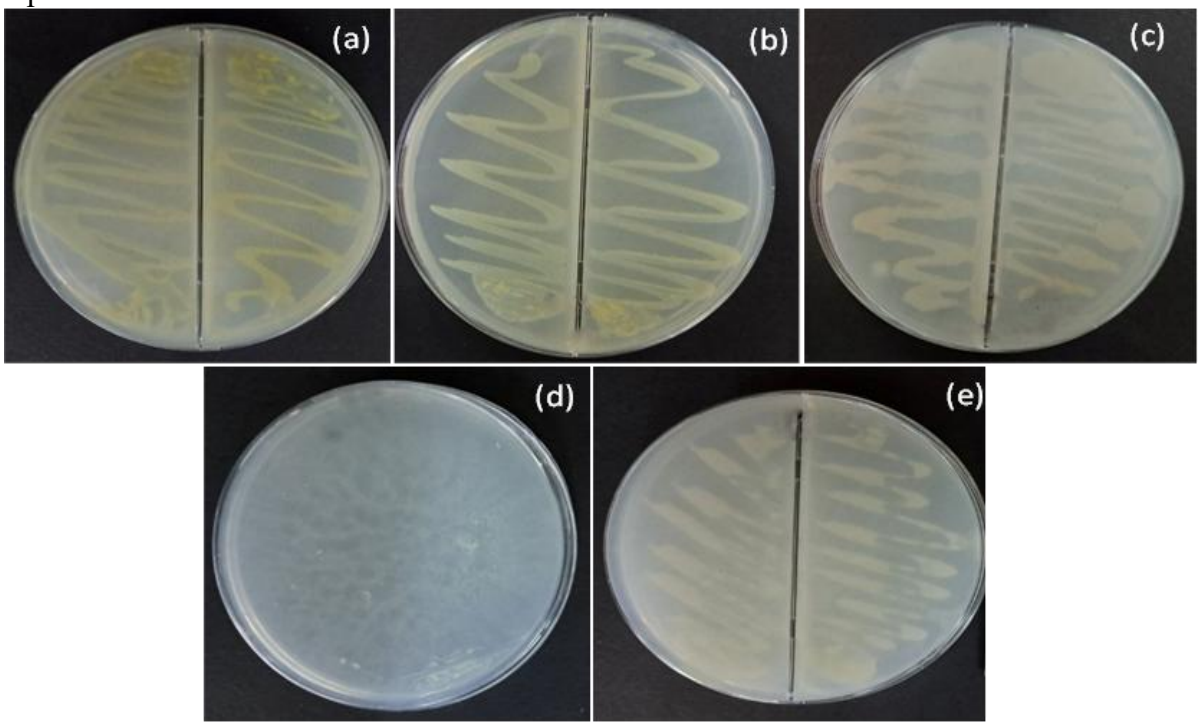

Figure 3. shows the effect of different salinities (NaCl) on bacterial strains; (a) effect of 0.5% NaCl on WD 50, (b) effect of 1% NaCl on WP 55, (c) effect of 4% NaCl on Xa 70, (d) and (e) showing the effect of 6% NaCl on different bacterial strains; (d) showing sensitivity of Ha 92 strain to high saline of 6% NaCl while, (e) showing tolerance of strain QC 95 to 6% NaCl

RESULTS AND DISSCUSSION

Nodules were collected for three wild chickpeas from sites; Sartaki-bamo, Penjween, Dere and three cultivated chickpeas from Qasre, Harir and Xarpane. A total of 30 endophytes bacteria were selected from

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chickpea root nodules. All the isolates were found to be gram negative due to obtained red the colonies in most isolates were circular-regular and entire. Out of 30 isolates, 24 (80%) were mucoid. Six isolates (20%) were non-mucoid. 15 isolates (50%) were growing better in 6.6-10.5 alkali pH, while 8 isolates (26.6%) were fast growing in acidity condition (pH 3.5-5.5). Out of the total 30 isolates 6 of them (20%) were grow better in neutral pH. It is recorded that 19 isolates (63%) had fast growing in 0-4% NaCl, while 9 isolated were sensitive to salinity media and 2 others were tolerant to 0-6% NaCl. It is also indicated that 11isolates (36.67%) were fast growing under temperature 25-40 ºC, while 17 (56.67%) had better growing under lower range of 15-35ºC, and 2 isolates grow faster under 15-40 ºC. In bromo thymol blue test it is recorded that 15 isolates (50%) were alkali/blue slow growing. These slow growing isolates produced white colonies, but in some cases the colonies were milky or yellow. Eight other isolates (26.6%) were growing faster in acid, that yielded yellow growing and 6 others (20%) produced green colony (Table 2). In present study, we have reported that all the 30 bacterial isolated were gram negative as they all showed red (pink) color, and being with the same morphology shape of their colonies. The shapes of the colonies in most isolates were circular or round except a few isolates which had puncti form and some were irregular. In most isolates surface and margin were smooth. It is realized that all of isolates that produced mucus showed tolerant to salt and high temperature stresses. Both fast and slow-growing isolates showed mucus production that ranged from high to intermediate with some isolates being dense and elastic and others diffuse and nonelastic. Mucus production probably represents a mechanism of rhizobia adaptation and endurance in hostile climatic and edaphic conditions. It prevents desiccation of the bacteria and helps them resist fluctuations under different stresses such as temperature, salinity, and acidity (Kenasa et al, 2014). This suggests that rhizobia isolate with high mucus production ability have highly competitive advantage in the initial infection, colonization, and root nodules (pink) color and all isolated have same morphology shape of colonies, the shapes of formation (Giongo et al, 2018). High soil temperature is one of the critical factors to prevent the development of a symbiotic association between the host plant and micro symbiont especially in arid and semi-arid regions (Ondieki et al, 2017). Optimum temperature for the growth of majority of rhizobia, is ranged from 28 to 31ºC and some of them are unable to grow at 38ºC (Lacerda-Júnior et al, 2019). In the current study we have found that 17 bacterial isolates in total 30 (56.67%) have ability to grow in a wider temperature range (15-35 ºC), other 11 isolates (36.67%) had fast growth in 25-40 ºC and 2 (6.67%) of bacteria isolates were grow faster in 15-40 ºC. In general, thermotolerance was found in almost the bacterial strains understudy. This property could refer to the interaction of the bacteria with specific hosts and soil properties or the physicochemical properties of soil, that affect the success of such bacterial nodule formation (Mabrouk et al, 2018), or it might refer to genetic improvements of strains for better adaptation to a specific stress such as high temperature (Granada Agudelo et al, 2023). Temperature affects the survival of free rhizobia as well as the molecular interaction between host and rhizobia. Raverkar, Gupta (Raverkar et al, 2005; Goyal et al, 2021) reported that increasing the temperature to 35 °C resulted in a significant decrease in nodule number, which might be refer to photosynthesis reduction at high temperatures that itself reduced supplying rhizobia with necessary food source. Indeed, raising temperature also affect the microbial respiration rate (Goyal et al, 2021).

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Table 2. Morphological and physio-chemical characteristic of bacterial isolates obtained from root nodule of Chickpea

NO.	Isolate code	Place of collection	Annual or perennial	Date of collection For plant	Elevation	Soil type	Node size and morphology	Colony Color	Colony morphology & surface margin	Muco	Gram test	Tolerance range			Bromo thymol blue with medium colony color
												pH	Salinity (Nacl%)	Temp. (°C)	
1	WS1	Sartak	Annual	27/5/2021	1742.3 m	Silty clay	Small	Whtie	Circular/ entire	+	Gram -ve	Alkali/ 6.5- 10.5	0- 4	25- 40	Alkali/ blue slow colony
2	WS3	Sartak	Annual	27/5/2021	1742.3 m	Silty clay	Small	White	Circular/ entire	+	Gram -ve	Alkali/ 6.5- 10.5	0- 4	15-30	Alkali/ blue slow colony
3	WS6	Sartak	Annual	27/5/2021	1742.3 m	Silty clay	Small	White	Circular/ entire	_	Gram -ve	Neutral/ 7.0	0- 0.1	15-30	Green/ neutral colony
4	WS9	Sartak	Annual	27/5/2021	1742.3 m	Silty clay	Small	Yellow	Circular/ entire	+	Gram -ve	Acid/ 3.5- 5.5	0- 4	15-35	Acid/ yellow fast colony
5	WS11	Sartak	Annual	27/5/2021	1742.3 m	Silty clay	Small	Yellow	Circular/ entire	_	Gram -ve	Neutral/ 7.0	0- 0.1	15-30	Green/ neutral colony
6	WS12	Sartak	Annual	27/3/2021	1742.3 m	Silty clay	Small	Yellow	Circular/ entire	+	Gram -ve	Acid/ 3.5- 5.5	0- 4	15-35	Acid/ yellow fast colony
7	WS17	Sartak	Annual	27/5/2021	1742.3 m	Silty clay	Small	White	Circular/ entire	_	Gram -ve	Neutral/ 7.0	0- 0.1	15-30	Green/ neutral colony
8	WS18	Sartak	Annual	27/5/2021	1742.3 m	Silty clay	Small	Yellow	Circular/ entire	+	Gram -ve	Acid/ 3.5- 5.5	0- 4	15-35	Acid/ yellow fast colony
9	WS21	Sartak	Annual	27/5/2021	1742.3 m	Silty clay	Small	Yellow	Circular/ entire	+	Gram -ve	Acid/ 3.5- 5.5	0- 4	15-35	Acid/ yellow fast colony
10	WS25	Sartak	Annual	27/5/2021	1742.3 m	Silty clay	Small	Yellow	Circular/ entire	+	Gram -ve	Alkali/ 6.5- 10.5	0- 4	15-30	Alkali/ blue slow colony
11	WS29	Sartak	Annual	27/5/2021	1742.3 m	Silty clay	Small	Yellow	Circular/ entire	+	Gram -ve	Alkali/ 6.5- 10.5	0- 4	25- 40	Alkali/ blue slow colony
12	WS31	Sartak	Annual	27/5/2021	1742.3 m	Silty clay	Small	Yellow	Circular/ entire	+	Gram -ve	Acid/ 3.5- 5.5	0- 4	15-35	Acid/ yellow fast colony
13	WS46	Sartak	Annual	27/5/2021	1742.3 m	Silty clay	Small	White	Circular/ entire	-	Gram -ve	Neutral/ 7.0	0- 0.1	15-30	Green/ neutral colony
14	WS48	Sartak	Annual	27/5/2021	1742.3 m	Silty clay	Small	Yellow	Circular/ entire	+	Gram -ve	Acid/ 3.5- 5.5	0- 6	15-35	Acid/ yellow fast colony
15	WS110	Sartak	Annual	27/5/2021	1742.3 m	Silty clay	Small	White	Circular/ entire	_	Gram -ve	Neutral/ 7.0	0- 0.1	15-30	Green / neutral colony
16	WD40	Dere	Perennial	21/5/2021	1394.7 m	Loam	Medium & irregular	Yellow	Circular/ entire	+	Gram -ve	Alkali/ 6.5- 10.5	0- 4	25- 40	Alkali/ blue slow
17	WD45	Dere	Perennial	21/5/2021	1394.7 m	Loam	Medium & irregular	Yellow	Circular/ entire	+	Gram -ve	Acid/ 3.5- 5.5	0- 4	25- 40	Acid/ Yellow fast colony
18	WD47	Dere	Perennial	21/5/2021	1394.7 m	Loam	Medium & irregular	Yellow	Circular/ entire	+	Gram -ve	Alkali/ 6.5- 10.5	0- 2	25- 40	Alkali/ blue slow colony
19	WD50	Dere	Perennial	21/5/2021	1394.7 m	Loam	Medium & irregular	Yellow	Circular/ entire	+	Gram -ve	Alkali/ 6.5- 10.5	0- 2	15- 40	Alkali/ blue/slow colony
20	WD102	Dere	Perennial	21/5/2021	1394.7 m	Loam	Medium & irregular	Yellow	Circular/ entire	+	Gram -ve	Alkali/ 6.5- 10.5	0- 4	25- 40	Alkali/ blue/slow colony
21	WD112	Dere	Perennial	21/5/2021	1394.7 m	Loam	Medium & irregular	Yellow	Circular/ entire	+	Gram -ve	Alkali/ 6.5- 10.5	0- 4	25- 40	Alkali/ blue/slow colony
22	WP35	Penjween	Perennial	26/6/2021	1488 m	Silty loam	Medium & irregular	Yellow	Circular/ entire	+	Gram -ve	Alkali/ 6.5- 10.5	0- 4	25- 40	Alkali/ blue slow colony
23	WP55	Penjween	Perennial	26/6/2021	1488 m	Silty loam	Medium & irregular	Yellow	Circular/ entire	+	Gram -ve	Alkali/ 6.5- 10.5	0- 2	25- 30	Alkali/ blue slow colony
24	WP111	Penjween	Perennial	26/6/2021	1488 m	Silty loam	Medium & irregular	Yellow	Circular/ entire	+	Gram -ve	Alkali/ 6.5- 10.5	0- 4	25- 40	Alkali/ blue slow colony
25	WP107	Penjween	Perennial	26/6/2021	1488 m	Silty loam	Medium & irregular	White	Circular/ entire	+	Gram -ve	Alkali/ 6.5- 10.5	0- 4	25- 40	Alkali/ blue slow colony
26	Xa 70	Xarpane	Annual	10/6/2021	787.4 m	Silty loam	Small	White	Circular/ entire	+	Gram -ve	Alkali/ 6.5- 10.5	0- 4	15- 35	Alkali/blue slow colony
27	Xa 72	Xarpane	Annual	10/6/2021	787.4 m	Silty loam	Small	White	Circular/ entire	+	Gram -ve	Alkali/ 6.5- 10.5	0- 4	15- 40	Alkali/ blue slow colony
28	Ha 92	Harir	Annual	12/6/2021	611.2 m	Clay loam	Small	White	Circular/ entire	+	Gram -ve	Alkali/ 6.5- 10.5	0- 4	15-35	Akali/blue slow colony
29	QC 93	Qasre	Annual	12/6/2021	1176 m	Clay	Small	White	Circular/ entire	_	Gram -ve	neutral/ 7.0	0- 0.1	15-30	Green/ neutral colony
30	QC 95	Qasre	Annual	12/6/2021	1176 m	Clay	Small	White	Circular/ entire	+	Gram -ve	Acid/ 3.5- 5.5	0- 6	15-35	Acid/ Yellow fast colony

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Variable growth rate was also identified for the isolated stains. It is identified that 50% of the isolates are growing better under range of alkaline condition (6.5-10.5), while 8 of the isolates (26.6%) had fast growing under acidity condition (pH 3.5- 5.5) and 6 others (20%) were grow in neutral p-H 7.0. It is reported that plant growth and soil nutrient availability and microbial activity are favored by a soil pH range of 5.5 – 8. The pH tolerance range is between 4.5 and 9.5 for Rhizobiaceae, however it has been reported that this range may be larger for rhizobia nodulating chickpea, since some isolates grew in a pH range from 4 to10 (Rai et al,2015, Jain et al,2020) these reports are in line with our finding. According to Maatallah, Sanjuan (Maataln et al, 2002), the pH tolerance range is between 4.5 and 9.5 for Rhizobiaceae, *Mesorhizobium sp*. develop an adaptive acid response during exponential growth upon exposure to sublethal acid condition (Rickert et al, 2000).However other studies showed that this range may be larger for rhizobia nodulating chickpea, since some isolates grew up to pH 10 (Rodrigues et al, 2006) and pH 4 (Rai et al, 2012). Excess of salinity adversely affects agriculture practices to influence soil structure and fertility, plant growth, yield, and microorganisms' activities (Tarolli et al, 2024). Soil salinity affects environmental health, in addition to agricultural production, and economic welfare (Negacz et al, 2022). It poses a significant thread to grain legume production in semi-arid and arid regions of the world (Rebecca et al, 2012). Rhizobia, endophytic non-nodulating bacteria and host plant are all affected by salinity, inducing ionic stress through osmotic stress due to the change in osmotic concentration around cells causing desiccation and water deficit (Coba de la Pena et al, 2012). Therefore, selection of salt tolerant isolates seems to be significant for chickpea cultivation in salt affected areas. In the current study, most of the bacterial isolates showed wide range of tolerance to salt stress. Their tolerance refer to the long-standing biological association with these rhizobia, especially in wild types, or it might be refer to the natural habitat of the endophytic bacterial population (Benjelloun et al, 2019). The isolates here gave alkaline and acidic results after bromothymol blue test, In the production of acidity or alkali of mesorhizobia, using bromothymol blue pH indicator, Half of the total isolates (50%) were with slow growing, producing blue colonies with the increased pH of the culture medium to alkali. While other 8 (26.6%) were produced acidic yellow colony and 6 (20%) isolated produced green colonies. It is previously reported that isolates yielded yellow, blue and green colony at %10 BTB test (Ali et al, 2019). Also, other investigations reported that rhizobia produce acidic and alkaline colonies (Singh et al, 2015; Singh et al, 2022). While others recorded the production of green color rhizobia at BTB which indicate that the isolate produce neutral colony, pH 7.0 (Brasca et al, 2018; Rahman et al, 2018). The current results line with previous reports producing slow blue colony with the increased pH of media to alkali, and also producing fast growing yellow with the decreased pH toward acidity (Muleta et al, 2021; Laurette et al, 2015). Fast growth characteristic of these isolates enables them to survive in soil when used as inoculum to this legume crop. The morpho cultural characteristics (rapid growth capacity and on Gram) confirmed the standard morpho cultural characteristics of Rhizobium species as reported by Somasegaran and Hoben (Somasegaran and Hoben, 2012). Nitrogen fixing bacteria are able to produce ammonia after 2–3 days of incubation, and changing the color of the medium from green to blue indicating an alkaline change due to ammonia production (Cordova-Rodriguez et al, 2022). While producing an alkaline reaction on yeast mannitol agar (YMA) and turning the medium blue due to bromothymol blue, means that the bacterium is producing a basic or alkaline substance. This suggests that it may not be metabolizing mannitol as a carbon source, or it may be metabolizing it in a way that basic is produced rather than acidic byproducts (Teja et al, 2022). Isolating bacteria from wild and cultivated chickpea from six different region of Kurdistan- Iraq, most of bacteria isolates were obtained from wild chickpea (Dere, Penjween and Sratak) and cultivated chickpea (Locally), after morphological and

biochemical test it is realized that most of the isolates are thermotolerant, salt tolerant, have normal growth in different pH acid and alkaline and produce microcity. There was similarity of bacterial isolates from Wild in Dere and Penjween which refer to (*Cicer oxydon*) nearly all of isolates are produce mucoid, temperature tolerant between 15-40 ^{o}C, produce alkali slow blue colony except S45 and all strains tolerant to 0-4 % NaCl stress except S47, S50 and S55 were slightly sensitive 0-2 % NaCl. While for wild chickpea of Sartak (*Cicer arietinum)*, most of bacterial isolates produce acidic fast yellow colony, non- mucoid and sensitive to salt stress. This may refer to the fact that wild-type plants have developed a symbiotic relationship with these rhizobia genera due to their genetic adaptations over time (Mendoza-Suárez et al, 2021). Also, the wild-type plants may have a long-standing biological connection with these rhizobia (11). There was a significant interaction between rhizobia isolates and plant hosts. This may be due to the host specificity between some rhizobia isolates and their hosts (Nabintu et al, 2019). The long-term association between these bacterial strains and chickpea plants indicates their adaptation to the local environment, allowing them to thrive under harsh conditions. These results highlight the potential of these bacteria to increase chickpea productivity in regions with harsh environmental conditions. Future studies could further explore the application of these bacteria in the development of sustainable agricultural practices, particularly in areas vulnerable to climate stress.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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عزل وتوصيف سلالات *Mesorhizobium* من عقد الحمص البري والمزروع في إقليم كردستان – العراق

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المستخلص

تم إجراء الدراسة للتحقيق في تكافل وتكوين العقد للبكتيريا من نوع Mesorhizobium في نمو الحمص (*Cicer arietinum* L.) تحت ظروف بيئية قاسية. تم جمع العقد من الحمص البري والمزروع في إقليم كردستان. تم عزل وتوصيف البكتيريا الداخلية Endophytes المرتبطة بجذورها. وُجد أن جميع العزلات الثلاثين هي بكتيريا سالبة الجرام، ذات مستعمرات ملساء ودائرية. كانت معظم العزلات (80٪) مخاطية، بينما لم تكن 20٪ كذلك وأنتجت مستعمرات خضراء على وسط البروموثيمول الأزرق. نصف العزلات (50٪) نمت بشكل أفضل في درجة حموضة قلوية (6.6–10.5)، بينما 26.6٪ ازدهرت في درجة حموضة حمضية (3.5–5.5)، وفضلت 20٪ منها درجة حموضة متعادلة. فيما يتعلق بتحمل الملح، نما 63٪ من العزلات بشكل جيد في تركيزات ملح تتراوح بين 0–4٪ NaCl، بينما تحملت 6.67٪ منها ما يصل إلى 6٪ NaCl. فيما يتعلق بدرجات الحرارة، نما 56.67٪ من العزلات بشكل أفضل في نطاق درجة الحرارة بين 15–35 درجة مئوية، بينما ازدهر 36.67٪ منها في نطاق 25–40 درجة مئوية. بعض العزلات كانت بطيئة النمو في الظروف القلوية، حيث أنتجت مستعمرات زرقاء، في حين أن العزلات سريعة النمو في الظروف الحمضية أنتجت مستعمرات صفراء. توفر هذه الدراسة رؤى حول قدرة هذه البكتيريا على التكيف تحت ظروف قاسية من الحموضة العالية، الملوحة، ودرجات الحرارة القصوى. وتقدم النتائج طريقًا لتحسين تحمل وإنتاجية الحمص في الأراضي غير خصبة في إقليم كردستان والمواقع المماثلة في جميع أنحاء العالم، من خلال استخدام العزلات المحلية من بكتيريا الرزوبيا المرشحة للبيئات القاسية.

الكلمات المفتاحية: ميزورايزوبيا، عقد الحمص، تكافل، سالبة الجرام، تحمل الملوحة، رايزوبيا المحلية.