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The Isolation and Characterization of Indigenous Rhizobacteria from Rhizosphere of *Phaseolus vulgaris* L. and their Potential Applications in Sustainable Agriculture

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Abstract:

This study aimed to isolate plant growth-promoting bacteria (PGPB) and characterize the isolates. A collection of soil from root rhizosphere of *Phaseolus vulgaris* L. cultured in Hawi Al-Kanissa region on the right bank of Tigris and Al-Qubba region on the left bank of Tigris River of Mosul City-Iraq was done. Eighteen rhizobacterial isolates were obtained, nine from each bank. Purification of rhizobacterial isolates was done and subjected to cultural and microscopic examination. Results revealed that the shape of the cells varied from short bacilli to bacilli and spherical. All the isolates were Gram-positive, with the exception of NR6 and NR7 which were negative for Gram stain. Results showed that all the isolates were motile except NR1, NR8, NR12 and NR16. Cultural emphasis showed extreme production of exopolysaccharide by the isolates NR9, NR12, NR13 and NR15. Biochemical test results revealed positive results for catalase, whereas negative results for oxidase, triple sugar iron, starch hydrolysis and citrate utilization. All the isolates were negative for urea test with the exception to NR8 and NR9. All the rhizobacterial isolates were positive for the indole test with the exception of NR11, NR12

and NR14. Sensitivity and resistance tests for the eight studied antibiotics revealed that three rhizobacterial isolates had resistance to three antibiotics or more. Test of tolerance of rhizobacterial isolates to different concentrations of sodium chloride revealed that these isolates were tolerant to this salt and the maximum tolerance reached 10 % (w/v) by the isolate NR3. It was concluded that the isolates varied in their cultural and biochemical characteristics as well as for antibiotic resistance and sodium chloride tolerance.

Keywords: Rhizobacteria, Biochemical, Antibiotic sensitivity, NaCl tolerance.

عزل وتشخيص الرايزوبكتريا المحلية من المنطقة الجذرية لنبات *Phaseolus vugaris* L. الفاصوليا

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الخلاصة:

تهدف هذه الدراسة الى عزل البكتريا المشجعّة على نمو النبات وتشخيص العزلات, حيث تم جمع تربة من المنطقة الجذرية لنبات الفاصوليا المزروعة في منطقة حاوي الكنيسة في الساحل الايمن وكذلك من منطقة القبة في الساحل الايسر من نهر دجلة من مدينة الموصل-العراق. تم الحصول على ١٨ عزلة رايزوبكتريا بواقع تسعة عزلات من كل ساحل. تم تنقية عزلات الرايزوبكتريا والقيام بالفحص الزرعي والمجهري واظهرت النتائج ان شكلها تراوح ما بين العصوي القصير الى العصوي والكروي وإنها موجبة لصبغة كرام ماعدا العزلتين NR6 وNR7 حيث كانتا سالبتين لصبغة كرام. كما أظهرت النتائج ان جميع العزلات متحركة ما عدا أربعة عزلات وهي: NR1 وNR8 وNR12 وNR16 حيث كانت غير متحركة. واظهرت نتائج الفحص الزرعي ان أربعة عزلات وهي: NR9 وNR12 وNR13 وNR15 كانت غزيرة انتاج السكر المتعدد الخارجي. وكما اظهرت نتائج الاختبارات الكيموحيوية انها موجبة للكاتاليز وتباينت نتائج اختبارات الاوكسيديز وثلاثي السكر والحديد وتحلل النشأ واستهلاك السترات. جميع العزلات كانت سالبة لاختبار اليوريا ما عدا العزلتين NR8 وNR9 وجميع العزلات سالبة لاختبار الاندول. أظهرت تجربة الحساسية والمقاومة لثمانية مضادات حيوية ان ثلاثة عزلات كانت مقاومة لثلاثة مضادات حيوية او أكثر. واظهرت تجربة تحمّل كلوريد الصوديوم لتراكيز مختلفة ان هذه العزلات كانت متحمّلة لهذا الملح ووصل اقصى التحمّل الى ١٠ % (وزن/حجم) في العزلة NR3. استنتجنا ان العزلات متنوعة في صفاتها المزرعية والكيموحيوية كما في الحساسية للمضادات الحيوية وتحمّلها لمح كلوريد الصوديوم.

الكلمات المفتاحية: رايزوبكتريا, الكيموحيوية, الحساسية للمضادات الحيوية, تحمّل كلوريد الصوديوم.

1. Introduction:

The microbiome-rhizosphere of leguminous plants constitutes a critical interface for nutrient exchange, disease suppression, and plant-microbe interactions. Research works indicate that legume roots selectively recruit beneficial bacteria through the secretion of

phenolic compounds, flavonoids, as well as organic acids, which act as chemoattractant for symbiotic. Rhizobia and other plant-growth-promoting rhizobacteria (PGPR) [1]. The microbial diversity within this area is shaped by plant, soil characteristics, and agricultural nutrients, making it a key factor in sustainable crop yield [2].

The mutualistic relationships between legumes and rhizobacterial rhizosphere extend beyond nitrogen fixation, encompassing induced systemic resistance (ISR) against any pathogens and improved biotic and abiotic stress tolerance [3]. Advances in metagenomics and microbial ecology have deepened our understanding of these interactions, paving the way for microbiome-based agricultural innovations [4]. Thus, studying the bacterial communities in the legume rhizosphere not only enhances our knowledge of plant-rhizobacterial symbiosis, but also offers practical solutions for eco-friendly cultures.

Plant Growth-Promoting Bacteria (PGPB) play a pivotal role in enhancing the productivity of leguminous crops, including green beans (*Phaseolus vulgaris* L.), through mechanisms such as biological nitrogen fixation, phytohormone production, and phosphate solubilization. These bacteria colonize the rhizosphere, forming symbiotic relationships that improve nutrient uptake and stress tolerance [5].

The rhizosphere rhizobacteria found in rhizosphere of *Phaseolus vulgaris* L. are highly dynamic, influenced by soil type, climatic conditions, and agricultural practices. Metagenomic studies reveal that some genera like *Rhizobium* and *Pseudomonas* spp. dominates this niche, contributing to nitrogen fixation and pathogen suppression [6].

2. Materials and methods:

2.1 Isolation of rhizobacteria:

Isolation of rhizobacterial bacteria was done according to Liu *et al.* [7] The field where the leguminous plant green beans is grown was selected, as this plant has been grown in the field on the right side for 48 years in the Hawi Al-Kanisa area and for a period of up to 50 years on the left side in the Al-Qubba area, taking into account that the soil is moist and not dry, as the plant was gently uprooted and shaken to exclude soil with weak adhesion to the root group, as the remaining soil attached to the root was chosen for isolation of rhizobacteria.

An amount of 1-2 grams of soil was taken from the upper root zone and 1-2 grams from the lower zone of the root of leguminous plant. The serial decimal dilution method is a core technique for assessing microbial density in soil. A 1-gram soil sample is mixed with 9 mL of

distilled water to achieve a 10^{-1} dilution. Subsequent dilutions were 10^{-2} to 10^{-6} are prepared by transferring 1 mL of the prior dilution into 9 mL of distilled water. Each dilution was then cultured on the appropriate medium and incubated [8] .

2.2 Cultural and microscopical emphasis:

Colony colour appearance and ability for the production of exopolysaccharide were recorded after culturing the 18 purified colonies on suitable solid medium and incubation for 1-3 days in an incubator at 30 °C. Cell shape and Gram staining were examined under a microscope with 1000x power.

2.3 Biochemical tests for rhizobacterial isolates:

Biochemical tests for isolating soil isolated rhizobacteria from rhizosphere of common bean were conducted and included the following tests: motility [9], urease [10], starch hydrolysis [11], catalase [12], Kovacs (indole) [13], citrate utilization [14], oxidase [15], triple sugar and iron test [16].

2.4 Intrinsic antibiotic resistance of rhizobacteria:

Antibiotic resistance testing of soil bacterial isolates was performed to determine the growth of plant growth-promoting bacterial isolates in the presence of the following antibiotics (µg/ml): Nitrofurantoin (N), 100; Ciprofloxacin (Cip), 10; Erythromycin (E), 10; Lincomycin (L), 10; Chloramphenicol (C), 10; Ampicillin (Am), 25; Clindamycin (Da), 10 and Amikacin (Ak), 10.

These antibiotics were produced by Turkey Bioanalysis. Using a cotton swab, the tested rhizobacterial isolates were spread onto Mueller-Hinton (M.H.A.) agar medium before introducing antibiotic discs. Using sterile forceps, liquid fresh growth was spread onto the medium, and cultured for 24 hours at 30 °C. The presence of a transparent halo surrounding the antibiotic discs was used to identify rhizobacterial isolates for sensitivity and resistance against different studied antibiotics [17,18].

2.5 Tolerance of rhizobacterial isolates to different concentrations of sodium chloride:

To emphasize the ability of isolated rhizobacteria for tolerance, the following concentrations of sodium chloride: 2.0, 4.0, 6.0, 8.0 and 10 % (w/v), a nutrient agar plate was supplanted the mentioned concentrations of sodium chloride inoculated with the same concentration of inoculum which equal 0.5 value on O.D. 600. [19]

3. Results:

The isolation and characterization of rhizobacteria from rhizosphere of roots of common bean (*Phaseolus vulgaris* L.) cultured in right bank (Hawi Al-Kanissa) and left bank (Al-Qubba) of the Tigris river in Mosul city of Iraq. A total of six rhizobacterial isolates were isolated and purified. Table (1) below shows microscopic and cultural characteristics of the isolated rhizobacteria. All the isolates with exception with NR6 were Gram-positive and positive for the motility test with exception to NR8 which were non-motile. Cell shape was varied from spherical to short rod to rod (Table 1). Colony colour was different for the six isolates as follows: yellow, orange, white and milky white.

Table 1: Cultural and microscopical examination of rhizobacterial isolates.

Isolate No.	Gram stain	Motility	Cell shape	Colony colour	EPS production
NR4	+	+	Spherical	Yellow	-
NR6	-	+	Short rod	Orange	-
NR8	+	-	Spherical	White	-
NR9	+	+	Short rod	Milky white	+
NR13	+	+	Rod	Orange	+
NR14	+	+	Rod	Orange	-

EPS: Exopolysaccharide.

Biochemical characterization tests (Table 2) revealed that all the isolates were positive for catalase and negative for indole tests. Oxidase test result revealed that NR4, NR6 and NR9 isolates were positive for this test and the rest isolates were negative for oxidase test. Triple sugar iron results were positive with the exception with NR13 which was negative for this test. Only two rhizobacterial isolates were positive for the urea test, i. e., NR8 and NR9 isolates, whereas the other isolates were negative for this test. Five of the six isolates were able to hydrolyze the starch and the rest isolate was negative for this test. Two isolates NR1 and NR14 showed the ability to utilize citrate, whereas other isolates were unable to utilize citrate as sole carbon source.

Table 2: Biochemical tests of rhizobacterial isolates.

Isolate No.	Catalase	Oxidase	TSI	Urea	Strach hydrolysis	Indole	Citrate utilization
NR4	+	+	+	-	+	-	+
NR6	+	+	+	-	+	-	-

NR8	+	-	+	+	+	-	-
NR9	+	+	+	+	+	-	-
NR13	+	-	-	-	+	-	-
NR14	+	-	+	-	-	-	+

TSI: Triple sugar iron test.

Results of resistance and sensitivity to the eight different studied antibiotics revealed that the isolates NR4 and NR8 were resistant to Nitrofurantoin 100 µg/ mL. All six isolates were sensitive to Ciprofloxacin 10 µg/ mL. The maximum average inhibition zone was 43 cm with NR9 isolate and minimum of average inhibition zone was 25 cm with NR4 isolate. Only one isolate (NR4) was resistant to Erythromycin 10 µg/ mL, whereas the other isolates showed different average inhibition zones and a maximum reached to 30 cm by NR9 isolate and minimum 18 cm by the isolates NR6 (Table 3).

Isolates NR9 and NR12 were resistant to Lincomycin 10 µg/ mL. The maximum average inhibition zone was 30 cm by NR8 isolate and minimum average inhibition zone was 10 cm by NR6 isolate. All the isolates were sensitive to chloramphenicol 10 µg/ mL with the exception of NR4 which was resistant to this antibiotic. The maximum average inhibition zone was 42 cm by NR8 isolate and minimum was 10 cm by NR6 isolate. NR9 rhizobacterial isolate was resistant to Ampicillin 25 µg/ mL, and the rest of the isolates were sensitive to this studied antibiotic. A maximum average inhibition zone was 32 cm by the NR13 isolate and minimum average inhibition zone was 17 cm by the isolate NR8.

NR9 was resistant to Clindamycin 10 µg/ mL, the other five isolates were sensitive to this antibiotic. Maximum sensitivity was by the NR4 isolate which was 34 cm and the minimum sensitivity was 9 cm by the NR13 isolate. None any of the isolates showed ability for resistance against the antibiotic Amikacin 10 µg/ mL, i.e. all the isolates were sensitive to this antibiotic and the maximum of average of inhibition zone was 29 cm by the isolate NR13 and the minimum average inhibition zone was 20 cm by the isolates NR4 and NR8.

Table 3:Resistance and sensitivity of rhizobacterial isolates to different studied antibiotics (µ/mL).

Isolate No.	F 100	Cip 10	E 10	L 10	C 10	Am 25	Da 10	Ak 10
NR4	R	25	R	28	R	23	34	20

NR6	16	35	18	10	10	20	27	25
NR8	R	33	29	30	42	17	27	20
NR9	15	43	30	R	33	R	R	23
NR13	30	40	24	R	37	32	9	29
NR14	20	29	18	15	20	20	22	18

Each value is mean of triplicates.

Abbreviations: F: Nitrofurantoin; Cip: Ciprofloxacin; E: Erythromycin; L: Lincomycin; C: Chloramphenicol; Am: Ampicillin; Da: Clindamycin; Ak: Amikacin.

Tolerance of the rhizobacterial isolates against different studied concentrations of sodium chloride revealed high ability of the isolated rhizobacteria to tolerate this salt (Table 4). From this table it is clear that the isolates have extreme tolerance to 2.0 % (w/v) concentration sodium chloride. With 4.0 % (w/v) concentration isolates NR8 and NR9 showed less ability to tolerate this concentration. Most of the isolates revealed varied growth from weak to good to very good growth on 6.0 % (w/v) concentration and the isolate NR9 showed no signals of growth. With 8.0 % (w/v) concentration three isolates NR6, NR8 and NR9 showed no growth and three isolates were able to grow viz., NR4, NR13 and NR14. All the six isolates were not able to grow on 10 % (w/v) which considered high concentration. (Table 4).

Table 4: Tolerance of rhizobacterial isolates to different NaCl concentrations % (w/v).

Isolate No.	**Concentration of NaCl % (w/v)**				
	2.0	**4.0**	**6.0**	**8.0**	**10.0**
NR4	+++	+++	++	+	-
NR6	+++	+++	++	-	-
NR8	+++	++	+	-	-
NR9	+++	++	-	-	-
NR13	+++	+++	++	+	-
NR14	+++	+++	+++	++	-

+++: very good growth; ++: Good growth; + moderate to weak growth; -: no growth.

4. Discussion

Two rhizobacterial isolates (NR9 and NR13) were high exopolysaccharide producers. [20] isolated polysaccharide producer and heavy metal-tolerant local rhizobial isolates. [21] isolated and identified rhizobacteria and they concluded that the Tigris and Euphrates rivers are

important for habitat developments as they provide water for agriculture and then support a variety of ecosystem. Their results on phenotypic examination revealed differences in Gram staining, motility and cell morphology as well as colony characteristics among isolated rhizobacteria. The positive catalase activity in all isolates suggests their potential for oxidative stress mitigation in plants. [22] reviewed antibiotic resistance in plant growth-promoting bacteria and their introduction into the soil could serve as antibiotic resistance gens and the invasion may enter into the food chain. Understanding the antibiotic resistance in plant agricultural systems, particularly those that use biofertilizers, is crucial because we must determine whether applying these fertilizers to the soil has the potential to select ARGs that could have an adverse effect on human health [22]. Therefore, it is essential to comprehend the antibiotic resistance profiles of PGPR in order to assess potential dangers that could affect the health of humans and animals, such as the transfer of resistance traits to dangerous bacteria [17]. Antibiotic resistance is evidenced through general different mechanisms such as decreased permeability then preventing penetration of antibiotics. The other method is Efflux system pump, the antibiotic pushed out of the cell before reaching the target. Target evasion and antibiotic metabolism, i. e., alter the structure of antibiotic targets [23]. [19] tested 20 rhizobacterial isolates and screened them for their salt tolerance potential and they found they showed halotolence to sodium chloride. Osmolyte compounds such proline, trehalose and total soluble sugars are biosynthesized in response to osmotic stress which and then accumulated in the cytoplasm as known as compatible solutes play a potential role in tolerance of salts [24]. [25] pointed out such these bacteria can be introduced into the root plant system to augment their stress-tolerant potential without compromising their productivity and lead to safeguarding the ecosystem.

5. Conclusions

Plant Growth Promoting Bacteria (PGPB) play a vital role in life sustainability as long as the rhizobacteria improve soil mixture as well as many positive roles in nature generally. This study succeeded in isolating and characterizing the isolates from soil in rhizosphere of *Phaseolus vulgaris* L. in both banks of Tigris river in Mosul city of Iraq. These isolates have a genetic marker such as antibiotic resistance genes as well as genes of tolerance of sodium chloride. Another feature of some of these isolates is their ability to produce high of exopolysaccharide which is considered signal of interaction between two partners leguminous plant and microorganism. Recent studies revealed that bacterial exopolysaccharides have industrial and medical applications, so these four isolates have immense potential in the future.

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