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Biodiversity of Rhizobia That Nodulate *Melilotus indicus* L. in Egyptian Soils

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THE OBJECTIVES of this work were to describe the biodiversity and the phylogeny of the selected rhizobial isolates nodulating wild legume *Melilotus indicus* L. (*M. indicus*) from 14 different Egyptian soils. These isolates were characterized morphologically and physiologically on the basis of their tolerance to NaCl and pH. Furthermore, the DNA of each rhizobial isolate was analyzed by rep-PCR amplification fingerprinting using REP, ERIC and BOX A1R primers. Thirty seven rhizobial isolates were obtained from the root nodules of *M. indicus*. These isolates didn`t absorb Congo- red (CR) when incubated in dark; grew poorly, or not at all, on peptone glucose agar medium containing bromocresol purple (BCP) and acidified the medium suggesting fast-growing rhizobia. Five isolates tolerated NaCl up to 7%. Rhizobial isolates showed a wide diversity in their pH tolerance. Moreover, PCR with REP and ERIC primer pairs yielded multiple distinct DNA products for each isolate of size ranged from approximately 177 to 3773 bp and 200 to 2921 bp, respectively. BOX A1R primer did not reveal any polymorphism for the isolates. We can conclude that rhizobia isolated from *M. indicus* from Egyptian soils are both phenotypically and genetically diverse.

Keywords: Genetic Diversity, Legume Nodules, *Melilotus*, Rhizobia.

In fact, rhizobial distribution and survival in natural habitats are due to the impact of the environmental factors prevailing in such habitats and the existence of the proper host. Also, the existence of diverse rhizobia helps the host legumes to be adapted to many different habitats (Bala & Giller, 2006). While, the great diversity and the vast geographic distribution of the legumes shaped their distinct rhizobial populations and drove their diversification (Drew & Ballard, 2010). Therefore, the diversity of rhizobia present in certain ecosystems is a result of interactions between rhizobia, their host legumes, biotic and abiotic factors of the ecosystem (Yan *et al*., 2014).

In the *Rhizobium*-legume symbiosis, the process of N_2 fixation is strongly related to the physiological status of the host plant. Therefore, competitiveness and persistence of rhizobial strains are not expected to be expressed in full

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capacity for nitrogen fixation if restrictive factors, such as salinity, unfavorable soil pH, impose limitations on vigor and growth of the host legume. A given stress may also have more than one effect: *e.g.*, salinity may act as a water stress, which affects photosynthetic rate, or may affect nodule metabolism directly (Zahran, 1999). Such stresses suppress growth and symbiotic power of most rhizobia (Gálvez, 2005).

The different responses of rhizobial strains to stress factors could be considered as basic criteria for differentiation and identification of these bacteria (Zahran *et al.*, 2012). Regarding rhizobia, soil pH and salinity are the main ecological factors determining their diversity (Adhikari *et al.*, 2012). It is expected that the genetic variation for traits in nature reflect their adaptation to specific environments (Koornneef *et al.*, 2004).

Studying rhizobial biodiversity enables isolation of an effective isolates from wild legumes that could be used in reforestation programs. Also, it`s a good strategy to improve quality and productivity of leguminous crops (food and feed) when inoculated with such isolates (Zahran *et al.*, 2012). Rhizobia of wild legumes may have better traits such as phosphorous solublization, producing plant growth promoting (PGP) compounds as indole acetic acid (IAA) and/or hydrogen cyanide (HCN) and/or possessing antibiosis effect (Alikhania & Yakhchali, 2009 and Arora *et al.*, 2001). Also, being tolerant to different stressful conditions more than the specific rhizobia of the cultivated leguminous crops (Abdel-Salam *et al.*, 2010 and Zahran *et al.*, 2012). Such bacteria are very important from both economical and environmental points of view (Kesari *et al.*, 2013).

Several molecular techniques have been readily developed to examine rhizobial biodiversity. These include: RAPD-PCR (random amplified polymorphic DNA-polymerase chain reaction), PCR amplification of REP (repetitive extragenic palindrome), ERIC (enterobacterial repetitive intergeneric consensus) and BOX element or RFLP (random amplified length polymorphism) of amplified ribosomal genes (16S, 23S or 16S-23S inter generic spacer - IGS), and *nod* or *nif* genes sequencing (De Bruijn, 1992; Edulamudi *et al.*, 2015; Laguerre *et al.*, 1994 and Versalovic *et al.*, 1994). Also, amplified ribosomal DNA restriction analysis (ARDRA) of both 16S and 23S rDNA (Shamseldin *et al.*, 2005) have been used to study the variation that exist for rhizobial isolates.

In Egypt, wild legumes, such as *M. indicus* plants, are widely distributed through the Mediterranean costal region, the Nil Delta and the deserts where there is a remarkable diversity of such plant species. *M. indicus*, sometimes incorrectly written *M. indica*, is a yellow-flowered herb native to northern Africa, Europe and Asia, as forage and as a soil improver (Velázquez *et al.*, 2010). Nevertheless, little information is known about the diversity of the endophytic rhizobia associated with *M. indicus* plants and their importance to legume establishment and growth.

Accordingly, the present work aimed to describe the biodiversity and the phylogeny of endophytic rhizobia nodulating wild legume *M. indicus* from 14 different Egyptian soils.

Material and Methods

Plant sampling and nodules collection

Fourteen locations were selected to study endophytic rhizobial diversity that exists in root nodules of *M. indicus* (L.) All. These locations were pinpointed by GPS (Magellan GPS 310), which distributed from East to West Mediterranean sea coast and within Nile Delta, (Table 1). Plants were sampled at the flowering and/or fruiting stage from the 14 locations. The collected plants were identified according to Täckholm (1974) and Boulos (2000). Nodules were collected and preserved according to Somasegaran & Hoben (1994).

TABLE 1. Locations of the collected plants, date of collection, latitude, longitude, and isolates number (no)/location. Alex=Alexandria.

No	Locations	Date	Latitude	Longitude	Isolates no/ location
1	Abo Shnar	1/4/2014	N 31 17 01	E 34 09 94	1.2, 1.2*
2	Rafah	1/4/2014	N 31 08 67	E 33 51 58	8.1, 8.7
3	Arish	1/4/2014	N 30 54 12	E 32 24 09	9.1, 9.17, 9.19
4	Port Said	31/3/2014	N 30 42 27	E 32 16 12	4.8, 4.17, 4.21
5	Ismailia	13/4/2014	N 30 31 49	E 32 09 49	11.1, 11.2
6	Ismailia	31/3/2014	N 30 29 86	E 32 07 13	3.6, 3.12*
7	Kafr Al-Shaykh	19/4/2014	N 31 05 01	E 30 57 42	13.1, 13.2
8	Rasheed	19/4/2014	N 31 22 19	E 30 24 57	16.3, 16.4
9	40Km West Alex	20/4/2014	N 30 55 39	E 29 28 09	14.1, 14.2, 15.1, 15.2
10	55Km West Alex	14/4/2014	N 30 54 59	E 29 26 52	6.2
11	El Dabaa	14/4/2014	N 31 00 57	E 28 33 46	7.1, 7.9
12	Shebeen El-Kom (*Unifarm*)	4/4/2014	N 30 55 77	E 31 01 74	10.1, 10.11
13	El Sadat City (*Unifarm*)	7/3/2014	N 30 23 07	E 30 30 55	12.1, 12.2, 12.3, 12.4
14	Faiyum (*Tamya*)	25/4/2014	N 29 29 07	E 30 54 40	2.2, 2.15, 2.36, 2.4, 2.7, 2.8

Isolation and purification of rhizobia from nodules

Endophytic bacteria were isolated from root nodules according to Vincent (1970) onto yeast extract mannitol agar (YEMA) containing Congo red (CR). The well purified single colony was streaked onto peptone glucose agar plates containing bromocresol purple (*BCP*) to confirm rhizobial selection. Well isolated colonies were re-streaked onto YEMA for better purification then single colony was selected and streaked on YEMA slants containing 1g calcium carbonate per liter then stored at 4°C.

Growth characterization of rhizobial isolates

The morphological traits (Elevation, margin, transparency, color, diameter and texture) and the production of acid or alkali were recorded by growing the isolates respectively on YEMA supplemented with CR and on YEMA supplemented with Bromothymol blue (BTB) (Somasegaran & Hoben, 1994 and Shetta *et al.*, 2011). Gram-stain reaction of the different rhizobial isolates and cell morphology were examined (Vincent, 1970).

Physiological characterization of rhizobial isolates

Several experiments were performed in broth tubes or agar plates inoculated with an exponentially growing liquid culture. All the experiments were carried out in triplicate otherwise it will be indicated. Bacterial growth was quantified as 0 (no growth), 1 (low growth), 2 (medium growth) or 3 (high growth = the control) otherwise it will be specified.

NaCl tolerance

Rhizobial isolates were grown on YEM broth at different concentrations of NaCl ranged from 1 to 7% (w/v) to test their salt tolerance. Growth was determined after 72 h of incubation at 28-30°C by measuring the turbidity at 550nm using spectrophotometer (Metertek SP-850) (Singh *et al.*, 2008).

pH tolerance

The ability of isolates to grow in acidic or alkaline media was determined on YEMA Petri dishes, where pH was adjusted either to 4, 5, 6, 9, 10 or 11. Bacterial growth was recorded (from 0 to 3), after 4 days of incubation at 28-30°C, compared to the control that was adjusted to pH 6.8 (Beauregard *et al.*, 2004).

Molecular characterization of rhizobial isolates

DNA isolation and gel electrophoresis

DNA was isolated from rhizobial cells either grown on solid or liquid media. The Stewart & Via (1993) DNA isolation protocol adopted originally for plant tissues was adapted in this work for rapid extraction of small quantities DNA. The concentration and purity of DNA were estimated spectrophotometrically at 260 and 280 nm, respectively.

PCR amplification with REP and ERIC primers

REP and ERIC fingerprinting were performed with primers REP 1R-I and REP 2-I and ERIC 1R and ERIC 2, respectively (De Bruijn, 1992). The PCR reactions for REP and ERIC primers were carried out in 25 µl volume with the following modifications: 1 µl of each two opposing primers (50 pmol/µl) (Metabion international AG, Germany); 0.4 µl of 50 mM dNTP Mix (AllianceBio); 2.5 µl polymerase reaction buffer (10 x) "complete": 160 mM $(NH_4)_2SO_4$, 670 mM Tris-HCl pH 8.8, 0.1 % Tween-20, 25 mM $MgCl_2$ (BIORON GmbH); 0.8 µl (5 U/µl) Taq DNA polymerase (BIORON GmbH); 1.5µl of DNA 50 ng/µl and finally sterile milli-Q water to complete the volume.

The cycles used were as follows: 1 cycle at 95°C for 6 or 7 min, 30 cycles at 94°C for 1 min, at 40 or 52°C for 1 min, and at 65°C for 8 min; 1 cycle at 65°C for 16 min; and a final soaking at 4°C, for REP or ERIC primers, respectively.

PCR amplification with specific BOX A1R primer

The DNA of each bacterium was amplified by PCR with primer BOX A1R as described by Kaschuk *et al.* (2006).

DNA and the amplified fragments were separated on 1% agarose gel (BIO-RAD) at 85 V for 3 h. GeneRuler^{TM} 1Kb Plus DNA Ladder, ready-to-use, (Fermentas) was used as molecular marker. All the PCR reactions were carried out in an TC-96/T/H(a) BIOER TECHNOLOGY CO., LTD, thermal cycler and the amplified fragments were separated by horizontal electrophoresis SGE-020-02, C.B.S.*SCIENTIFIC (20 cm * 20 cm). Gels were stained with ethidium bromide, visualized under UV light (UVP, PhotoDoc-It, ^{TM} Imaging System Digital, UK) and photographed with a Canon Power Shot A720 IS camera. The gel photos were analyzed using UVI soft UVI band map windows Application Vll.ll.

Results

Isolation and purification of rhizobia from nodules

A total of 37 rhizobial pure isolates were selected from the endophytic bacteria that exist in the root nodules of *M. indicus* plants. They showed little or no CR absorption when incubated in dark. The rhizobial isolates grow poorly, or not at all, on peptone glucose agar medium containing BCP, heavy growth is indicative of contamination (Somasegaran & Hoben, 1994).

Growth characterization of rhizobial isolates

The colony shape of the obtained 37 isolates was either dome (30 isolates) or flat (seven isolates). The colonies were round with smooth edges. Thirty two isolates were opaque while five isolates were translucent. Colony color was milky (27 isolates), white (4 isolates), yellow (2 isolates) or watery (4 isolates) while colony size ranged from 0.1cm to 0.7cm in diameter. The texture varied from being not sticky (8 isolates), semi sticky (2 isolates) to sticky (27 isolates) colonies.

The green color of agar media (YEMA containing BTB) changed to yellow color as all the selected isolates acidified the medium assuming fast-growing rhizobia.

All the isolates were gram-negative being short rod in shape with pink color under light microscope.

Physiological characterization of rhizobial isolates

A number of experiments were carried out in order to characterize the rhizobia isolated from root nodules of *Melilotus indicus* (L.) physiologically. Such experiments will be used for further clustering to discriminate between the rhizobial isolates for their similarities as well as their natural variation.

NaCl tolerance

All isolates grew in the control cultures (28-30ºC, pH 6.8 and 0.01% NaCl). Fig.1 showed the frequency distribution of the rhizobial isolates grew at different NaCl concentrations. Most of the isolates were able to grow and showed good salt tolerance efficiency up to 3% w/v NaCl. While, 81%, 70.2% and 32.4% of the isolates could grow up to 4%, 5% and 6% NaCl, respectively. Isolates 9.1, 9.17, 10.11, 15.1 and 15.2 were able to tolerate a maximum concentration up to 7% NaCl.

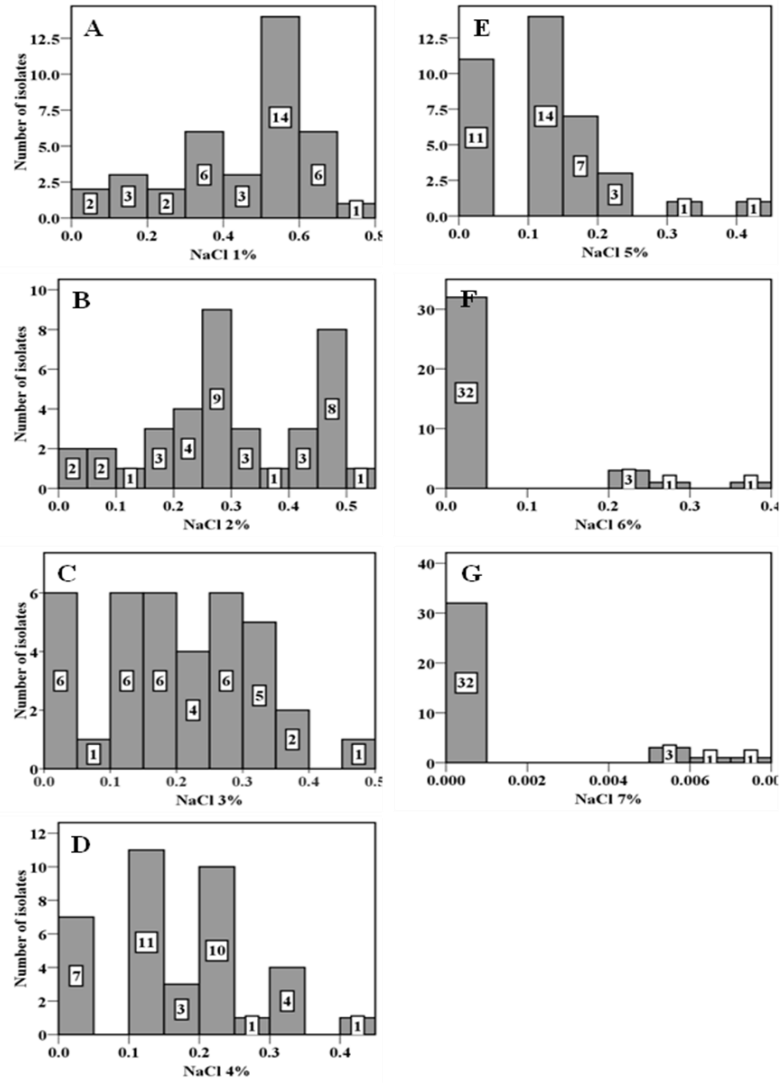

Fig. 1. Frequency distribution of the different isolates showing the effect of different NaCl concentrations.

When analyzed separately, the dendrogram obtained from numerical analysis of the isolates according to their tolerance to different NaCl concentrations showed a wide diversity among them (Fig. 2). All the isolates were placed in two distinctive major groups (group I and II). Group I differentiated into two subgroups; subgroup 1 include isolates (12.1, 12.2, 7.9, 12.4 and 7.1), that were able to grow and tolerate NaCl concentration up to 3% and few of them had the same origin. While, subgroup 2 include isolates (13.1, 14.2, 8.1, 13.2 and 12.3) that were able to grow and tolerate NaCl concentration from 3% to 5% and few of them had the same origin. Group II separated to two faraway subgroups; subgroup 1 and subgroup 2. The latter was divided into many subgroups, where isolate 6.2 was the most deviating one. Also, in this subgroup 2, isolates (2.7, 2.8, 2.15, 2.36, 2.2, 2.4 and 4.8) were closely clustered. Such isolates were able to grow and tolerate NaCl concentration up to 6% and had the same origin.

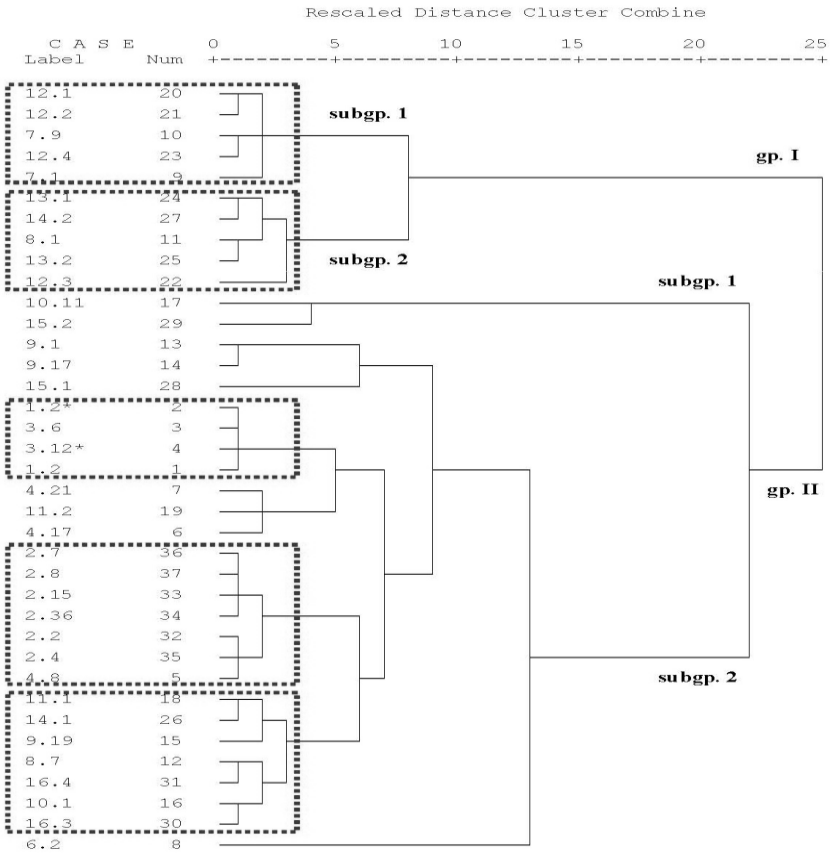

Fig. 2. UPGMA dendrogram of physiological relationships among the rhizobial isolates determined by NaCl tolerance. Rectangles indicating closely clustered groups (gp.) and/or subgroups (subgp.).

pH - tolerance

As shown in Fig. 3, the rhizobial isolates showed a wide diversity in their pH tolerance. All the isolates grew well at pH 6.8 being the control cultures. At pH 9, 89.2% of the isolates gave high growth while 10.8% of the isolates gave moderate growth. At pH 10, 70.3% and 2.7% of the isolates showed moderate and low growth, respectively. At pH 11, 68% of the isolates gave low growth. 86.5% and 70.3% of the isolates were unable to survive at pH 4 and 5, respectively. While, 13.5% of the isolates gave low growth at pH 4, and 16.2%, 5.4% and 8.1% of isolates showed low, moderate and high growth at pH 5, respectively.

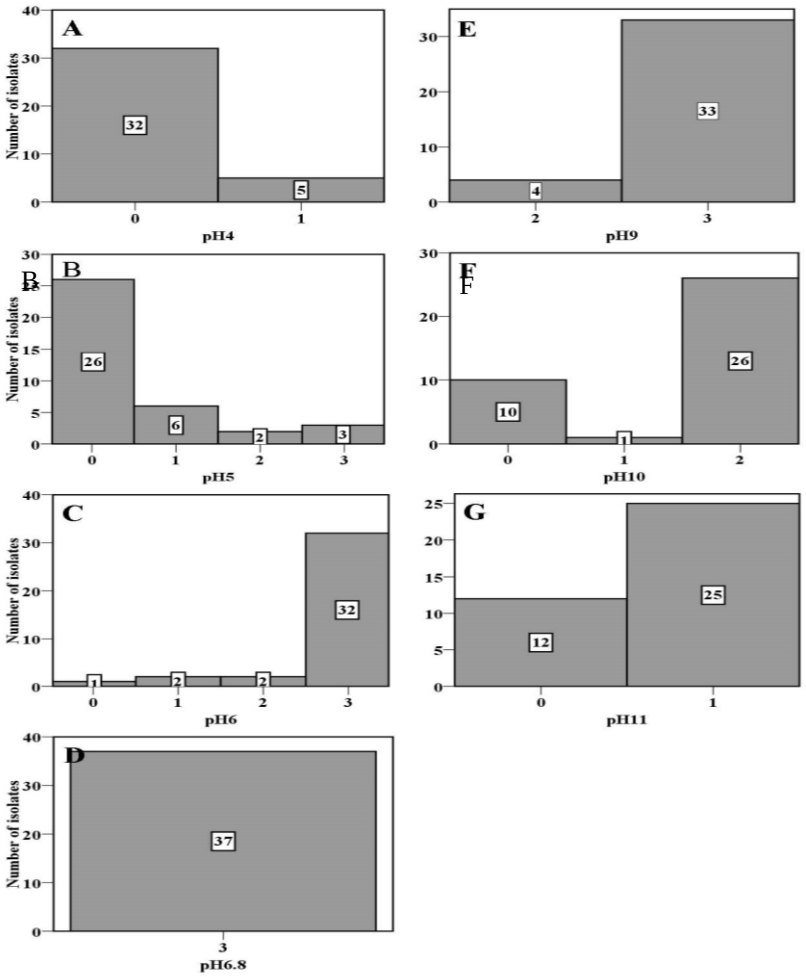

Fig. 3. Frequency distribution of the different isolates showing the effect of different pH.

Cluster analysis of the rhizobial isolates according to their tolerance to the different pHs showed a diversity among them (Fig. 4). Two isolates (7.9 and 12.4) were clustered separately in group II away from group I. The latter was subdivided into two subgroups, where subgroup 1 comprised of 7 clusters. Cluster 1 include isolates (2.7, 2.8, 1.2*, 2.36, 2.4, 2.2, 2.15, 14.2, 15.1, 7.1, 13.2, 4.8 and 4.17) that had isolates with the same origin and able to grow at pH values above 6.8 till 11. Cluster 2 comprised of five isolates (11.2, 13.1, 10.1, 10.11 and 11.1), that were able to grow at pH values from 5 to 11 and few of them had the same origin. Cluster 6 comprised of six isolates (9.19, 12.3, 3.6, 9.1, 9.17 and 3.12*) that had isolates with the same origin (few) and able to grow at pH values 6, 6.8 and 9 (Fig. 4).

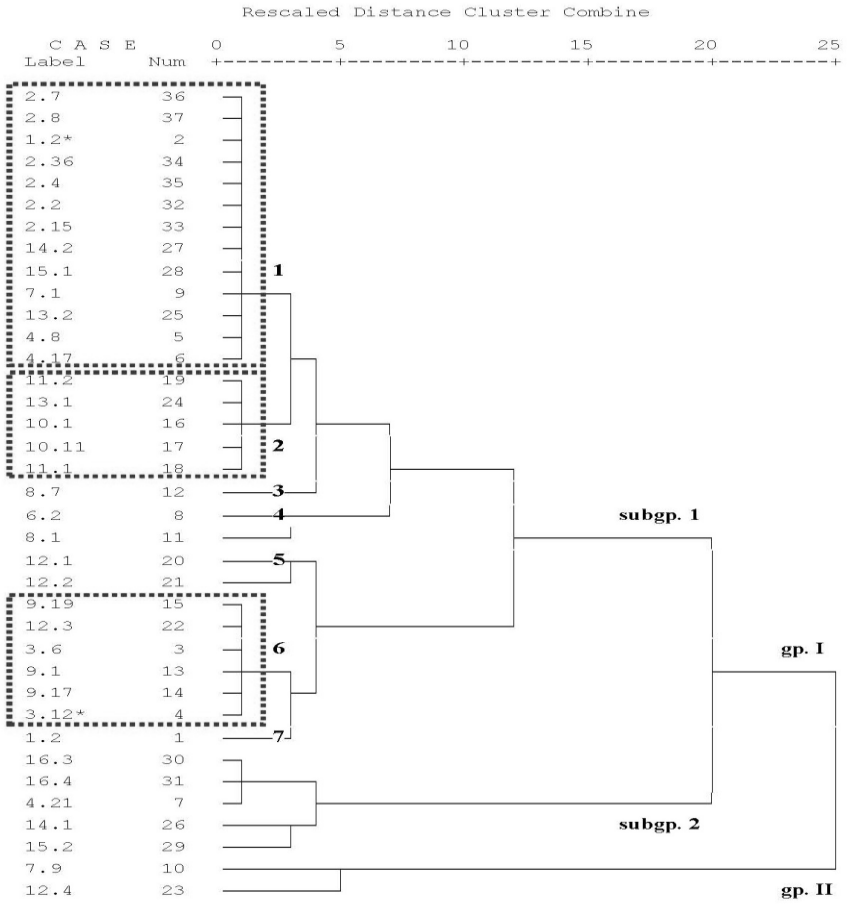

Fig. 4. UPGMA dendrogram of physiological relationships among the rhizobial isolates determined by pH tolerance. Rectangles indicating closely clustered groups (gp.) and/or subgroups (subgp.).

Molecular characterization of rhizobial isolates

Genomic DNA was extracted from the 37 rhizobial isolates. This DNA was used as a template for amplification of rep PCR primers (REP, ERIC and BOX). Such PCR products were used to discriminate between the isolates and to obtain a picture about the diversity and the natural variation that might exist between the rhizobial isolates.

PCR amplification with REP and ERIC primers

PCR with REP and ERIC primer pairs of DNA from the isolates yielded multiple distinct DNA products of size ranged from 177 to 3773 bp with the REP primer and from 200 to 2921 bp with the ERIC primer.

High resolution REP and ERIC PCR fingerprints of the 37 isolates were generated. Isolates (4.8, 7.1, 10.11, 11.1, 15.2 and 16.4) and (2.7, 2.15, 3.6, 7.1, 7.9, 8.1, 8.7, 10.11, 11.2, 13.2 and 16.4) did not produce a PCR amplification profile with REP and ERIC PCR respectively, while the remaining 31 and 26 isolates were resolved in distinctive profiles with REP and ERIC PCR, respectively.

REP- PCR analysis of the 31 isolates revealed a relatively high level of genetic diversity for isolate 12.2. On the other hand, isolates (3.6, 9.1 and 10.1), (9.19, 14.1 and 14.2;), (2.15 and 4.17) and (2.36, 2.4, 2.7 and 2.8;) showed 100% homology except 14.2 being 85% (Fig. 5).

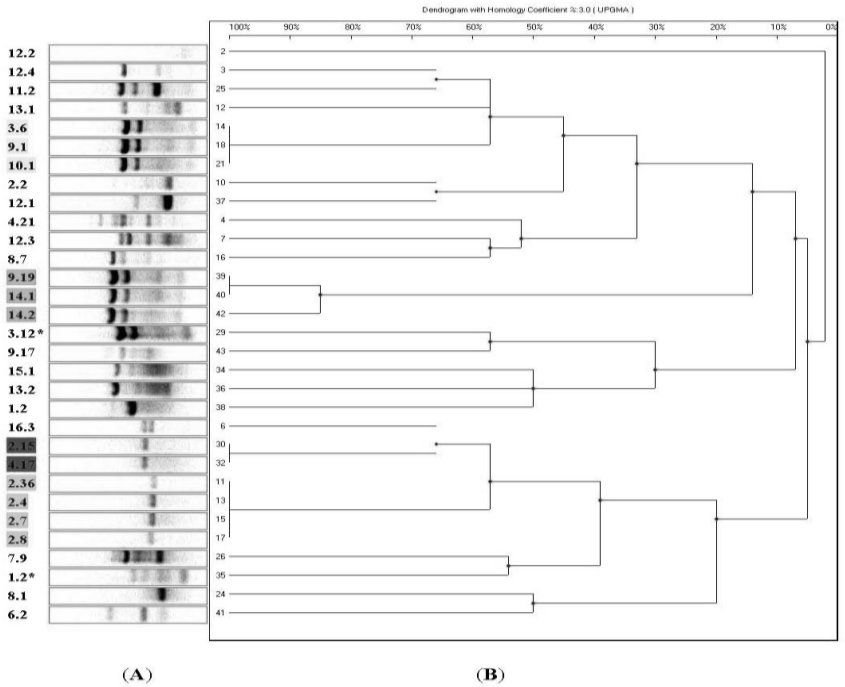

Fig. 5. Electrophoretic patterns for the different rhizobial isolates generated by rep-PCR using REP primer (A). Dendrogram of the 31 rhizobial isolates originated by UPGMA cluster analysis based on REP primer (B).

ERIC PCR analysis of the 28 rhizobial isolates showed 100% high similarity between isolates (12.2 and 12.3) and (2.2 and 2.36). While the dendrogram showed isolates (2.8, 3.12*, 15.1, 1.2, 14.1) that was closely clustered together with percentage of 65% similarity (Fig. 6).

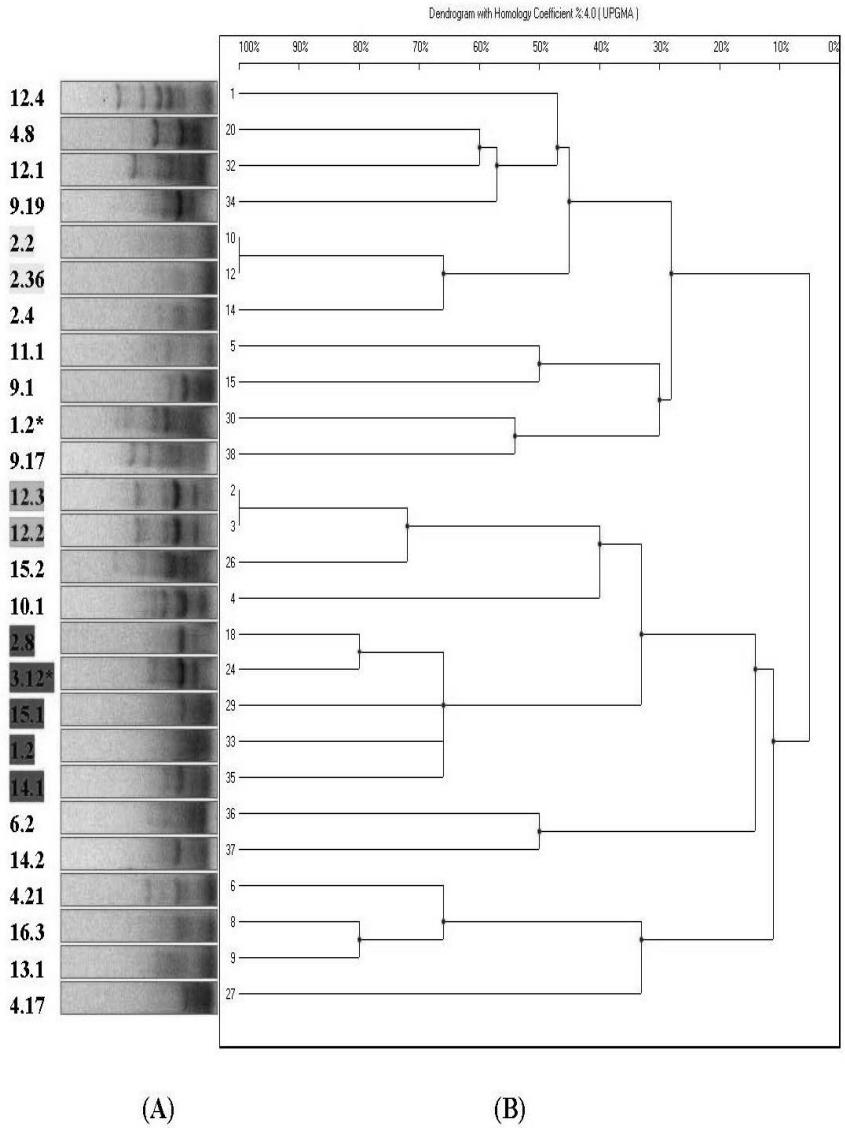

Fig. 6. Electrophoretic patterns for the different rhizobial isolates generated by rep-PCR using ERIC primer (A). Dendrogram of the 28 rhizobial isolates originated by UPGMA cluster analysis based on ERIC primer (B).

PCR amplification with specific BOX A1R primer

BOX A1R primer did not reveal any polymorphism in all isolates (data not shown).

Discussion

Colonies of the obtained isolates showed little or no CR absorption when incubated in dark and grow poorly on peptone glucose agar medium containing BCP. Therefore, it is designated as colonies of a typical *Rhizobium* species. This judgment coincides with that obtained by others (Deshwal & Chaubey, 2014 and Somasegaran & Hoben, 1994).

In this study, the colonies of the 37 rhizobial isolates showed large variation in their morphological traits similar to those obtained by Singh *et al.* (2008) and Zahran *et al.* (2012). Regarding the pH-reaction, the 37 rhizobial isolates showed an acid reaction assuming that these isolates are fast-growing rhizobia. In this respect, the obtained results coincide with that obtained by many workers (Baoling *et al.,* 2007; Hatice *et al.*, 2009; Somasegaran & Hoben, 1994 and Vincent, 1970).

Saline conditions may limit *Rhizobium*-legume association by affecting survival and proliferation of *Rhizobium* spp. in soil and rhizosphere; inhibiting the early stages of infection process; affecting root nodule development and reducing the host growth (Evans, 2015 and Graham, 1991). In the present work, most of the rhizobial isolates showed high capacity for salt tolerance as 26 and 5 out of 37 isolates could withstand up to 5% and 7% NaCl, respectively. This NaCl tolerance agreed with previous reports (Abdel-Wahab *et al.,* 2002; Al-Shaharani & Sheetta, 2011; Legesse & Assefa, 2014; Zahran *et al.,* 2003 and Zahran *et al.,* 2012). Nevertheless, other *Rhizobium* strains from arid saline areas were highly salt-tolerant and withstand high NaCl levels up to 5-10% (Abdel-Wahab *et al.,* 2002 and Zahran *et al.,* 2003).

The dendrogram obtained from the numerical analysis of NaCl tolerance of the 37 isolates showed a wide diversity among them. Only, few isolates grouped together as they were able to tolerate NaCl concentration up to 3% or 5% and few of them had the same origin.

Extreme pH can be a major factor limiting fast growing groups. Slight variation in pH of the medium might have significant effects on the growth of bacteria or organism (Adhikari *et al.,* 2012; Singh *et al.,* 2008 and Yan *et al.,* 2014). In this study, the majority of isolates could tolerate pH up to 10, while 25 out of 37 isolates gave poor growth at pH 11. On the other hand, at acidic conditions (pH 4 and 5), only 5 and 11 isolates could grow, respectively. Such results agreed with studies on rhizobial strains (Guerrouj *et al.,* 2013; Legesse & Assefa, 2014; Youseif *et al.,* 2014 and Zahran *et al.,* 2012), who showed that even pH 10 was not inhibitory to rhizobial strains and these strains were sensitive to acidic conditions (pH 3.5-4.0).

Both REP- and ERIC-like sequences (elements) are present in the genomes of gram-negative soil bacteria, such as rhizobia (De Bruijn, 1992). In the present work, both REP and ERIC-PCR profiles enabled strain differentiation and demonstrated a considerable degree of genetic diversity among the rhizobial isolates. The isolates were clustered on the bases of fingerprinting similarity where few of them had the same origin. Also, our results support the conclusion of De Bruijn, (1992), Edulamudi *et al.* (2015) Evans (2015) and Laguerre *et al.* 1994) that the REP and ERIC PCR could become a powerful tool for the molecular genetic analysis of bacteria and for bacterial taxonomy. It allows the fingerprinting of individual genera, species, and strains and could help to determine phylogenetic relationships. Also, these findings are in agreement with the results obtained by other authors who studied diversity among natural rhizobial populations in different world regions (Adiguzel *et al.*, 2009 and Granada *et al.*, 2014).

It could be concluded that rhizobia isolated from root nodules of *M. indicus* from Egyptian soils are both phenotypically and genetically diverse. Utilization of both salt and pH tolerant rhizobial isolates may contribute to the reclamation of salt affected soils which occupy large worldwide areas.

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تنوع الريزوبيا المعزولة من نبات الحندقوق المر في الأراضي المصرية

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يهدف البحث إلى وصف التنوع البيولوجي لبكتريا الريزوبيا المعزولة من نبات الحندقوق المر من 14 مكان مختلف من الأراضى المصرية على المستوى الفسيولوجى و الوراثى بالإضافة إلى المظهر الخارجى لهذه العزلات.

حيث تم وصف هذه العزلات بناءا على مظهرها الخارجى وفسيولوجيا على أساس تحملها لدرجة الملوحة ودرجة الأس الهيدروجينى. وعلاوة على ذلك تم تحليل الحامض النووى لهذه العزلات عن طريق استخدام علامات وراثية مختلفة باستخدام rep-PCR .

تم الحصول على 37 عزلة ريزوبيا من العقد الجذرية لنبات الحندقوق المر. وإستنادا إلى التجارب الفسيولوجية كانت جميع العزلات سريعة النمو ، بينما كانت سبع عزلات فقط متحملة لدرجة ملوحة حتى 7% من كلوريد الصوديوم. وأظهرت عزلات الريزوبيا تنوعا واسعا فى تحملها لدرجات الأس الهيدروجينى المختلفة. علاوة على ذلك فإن دراسة تحليل الحامض النووى لهذه العزلات عن طريق استخدام علامات وراثية مختلفة باستخدام rep-PCR أنتج العديد من قطع الحامض النووى المتميزة فى الحجم والتى تتراوح ما بين 177 إلى 3773 قاعدة فى حالة الـ REP ، بينما تتراوح ما بين 200 إلى 2921 قاعدة فى حالة الـ ERIC . لم تظهر أية فروق باستخدام BOX A1R كدلالة وراثية مع جميع العزلات.

لذلك يمكننا أن نستنتج أنه يوجد تباين و تنوع في المظهر الخارجي والوراثي بين عزلات الريزوبيا المعزوله من نبات الحندقوق من الأماكن المختلفه في مصر.