FACULTY OF AGRICULTURE

Minia J. of Agric. Res. & Develop.
Vol. (34), No.1, pp. 137-147, 2014

STUDYING GENERAL CHARACTERISTICS AND EFFECTIVENESS OF *RHIZOBIUM* ISOLATED FROM DIFFERENT LEGUMINOUS PLANTS

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ABSTRACT

A total of 25 rhizobia isolates were recovered from different growing sites at El-Minia governorate. Isolates were considered fast-growing rhizobia depending on: growth rate, colony morphology, Gram stain, Congo red absorption, acid/alkali production, motility, casease test, gelatin hydrolysis, starch hydrolysis, and are finally confirmed as rhizobia by the nodulation test. All isolates were mucoid, circular and motile and didn't absorb Congo red dye. They differed from raised to high convex, from white to watery, and from opaque to translucent. *Rhizobium* strains are Gram negative rods. All produced acids from mannitol. None could hydrolyzestarch, casein and gelatin. Pot experiment indicated that all isolates could nodulate their corresponding plants but their effectiveness differed.

Keywords: *Rhizobium*, general characteristics, nodulation

INTRODUCTION

Nitrogen is needed by all organisms for the synthesis of protein, nucleic acid and other nitrogen containing compounds. Despite its abundance, however, no eukaryote is able to make direct use of nitrogen, a molecular gas. Instead the nitrogen must be fixed (combined) with other elements such as oxygen and hydrogen. Conversion of nitrogen to ammonia by microorganisms, is a process called biological nitrogen fixation (BNF) (Talaro, 2009). BNF is the second most important biological process on earth after photosynthesis (Sylvia *et al*. 2005). BNF is brought about by two types of organisms: non symbiotic and symbiotic. Symbiotic BNF is illustrated by members of the genus *Rhizobium* with the roots of leguminous plants (Tortora*et al*. 1982).Rhizobia infect host plants, and induce root- or stem-nodules (Zahran, 2010). The rhizobial bacteria are specific for a particular leguminous species (Fauvert and Michiels. 2008). Rhizobia-legume symbiosis is the most efficient system which accounts for almost 40% of all biologically fixed nitrogen (Yadav, 2008).

Bacteria forming nitrogen-fixing symbiosis with legumes were classically named “rhizobia”. They belong to the α-

and β- subdivisions of Proteobacteria(Sawada *et al.* 2003) and currently consist of about 76 species spanning over 15 genera, namely, *Rhizobium, Allorhizobium, Azorhizobium, Blastobacter, Bradyrhizobium, Burkholderia, , Devosia, Ensifer* (formerly *Sinorhizobium*), *Mesorhizobium, Methylobacterium, Shinella* (Medeot*et al.* 2010), *Herbaspirillum*(*H. lusitanum*nodulating*Phaseolus vulgaris*)(Valverde*et al.* 2003), *Ochrobactrum* (Zurdo-Pin˜eiro*et al.* 2007), *Phyllobacterium* (Valverde*et al.* 2005) and*Ralstonia (*Later*Cupriavidus)* (Chen *et al.* 2001). Genus *Rhizobium* contains about 33 species (Vela´zquez*et al.* 2010).

Our study aimed at isolation, characterization and determination of effectiveness of *Rhizobium* due to its economic value and the need to use it as a substituent to chemical fertilizers that threat health.

MATERIALS AND METHODS

1. Isolation of Rhizobium *from selected plants*

Healthy, unbroken, firm and pink colored nodules were selected from 45-90days old legume plants (*Viciafaba, Pisumsp, Lens sp, Trigonellasp&Trifoliumsp*). Adhering soil particles were removed by washing root system under running tap water. Nodules were removed and transferred to 0.1% $HgCl_2$ or 3-5% H_2O_2 for 3 min for surface sterilization. The nodules were repeatedly washed in sterile water for 3-4 min, to get rid of the sterilent, then were placed in 70% ethyl alcohol for 3 min and were washed with sterile water thoroughly and crushed with sterile forceps. The resulting suspension was streaked on Yeast Extract Mannitol Agar (YEMA) medium containing (0.0025% Congo-red dye (CR)), incubated at 28 +/- 1˚C for 3-5 days (depending upon growth rate)(Vincent, 1970).

2. Purification of isolates

Purity was checked by repeatedly streaking single colorless or white colonies on agar plates of YEMA-CR.The inoculation needle was flamed after streaking the half of each plate, to reduce number of viable cells and thus single colonies could be obtained. Growth on plate was periodically observed to record diameter of isolates. Isolates of *Rhizobium* were designated R, followed by serial numbers.

3. Preservation of isolates

Slant cultures can be stored for 2-3 years or longer at 2^0C (Vincent, 1970). Long-term storage at -20^0C or -80^0C is recommended in the freezer (Kuykendall *etal.*2005).

4. General characteristics of Rhizobium

Colony morphology: Colour, mucoidty, transparency, elevation, diameter, and borders were evaluated by streaking a loop of the initial bacterial culture on YEMA-CR plates and allowing the bacteria to grow at 30°C for 5 days.

Gram staining: It was performed formicroscopic studyof *Rhizobium*as described by (Vincent, 1970).A drop of bacterial culture was taken and thin smear was prepared on a glass slides and stained.

Acid or alkali production test: It was carried out by growing isolates on YEMA medium containing bromothymol blue (BTB) (Somasegaran and Hoben. 1994) as pH indicator (5ml/L) of a stock solution. After incubation, yellow color indicates acid production, while blue color means alkali.

Motility test: It was done by growing cultures insemi-solid YEMA containing a dye, TTC (Triphenyl tetrazolium chloride). 10 ml of 1%filter-sterilized TTC solution

were added to 1 L of melted YEMA (semi-solid) after autoclaving. 5 ml aliquots were dispensed aseptically into sterile tubes and cooled upright in racks. As bacteria grow in, the dye is absorbed into the bacterial cells where it is reduced to the insoluble red-colored pigment formazan (MacFaddin, 1972).The inoculating needle (straight needle without loop) was used to make a single stab down the middle of a tube of semi-solid agar. Tubes of agar were incubated at 30°C for 6 days. The results were interpreted. If the zone of growth spreads horizontally from the inoculation, then the organism is motile.Non-motile organisms don't spread out.

Casease Test: Skim milk agar was used to test the ability of an organism to produce an exoenzyme, called casease that hydrolyzes casein(Larone, 1993). Plates wereinoculated with a loopful of each culture and incubated at 30 ^{0}C for 5 days. If an organism can break down casein, a clear halo will appear around the areas of growth.

Gelatin hydrolysis: The test was performed to determine capability of microorganisms to produce gelatinase enzyme (Aneja, 2003). The actively grown cultures were inoculated in nutrient gelatin medium tubes and grown for 48 h. On subjecting the growing culture to low temperature treatment at 4°C for 30 min or more, the cultures which produce gelatinase remains liquefied while others due to presence of gelatin becomes solid.

Starch hydrolysis: The test was performed to determine capability of microorganism to use starch as carbon source (De Oliveira*et al*. 2007). Starch agar medium was inoculated with *Rhizobium* isolates, incubated and analyzed. Iodine test was used to determine capability of isolates to use starch.Formation of blue color indicated non-utilization of starch and vice versa.

Pot experiment: (1) Inoculation of plants

Pot experiment was conducted in the greenhouse of Minia University Faculty of Science, with three replicates for each treatment. The cultivation process was carried out during the 2011-2012 (for winter plants), using plastic pots of 15 cm diameter x 20-cm-deep pots containing 1200 g of autoclaved soil. The soil used was collected from the surface 15 cm layer of the botanic garden of Minia University Faculty of science. Sterilization process was repeated twice. Physical and chemical analyses of the used soil were determined in the service laboratory for soil, plant and water analysis in the Faculty of Agriculture, Minia University.Physical and chemical analyses of the used soil areindicated in Tables (1& 2).

Pots were irrigated a day before planting.2 days old liquid cultures were used as inocula (10^{9}cfu/ml).25 ml inoculum was applied for each pot. Each isolate was inoculated into the corresponding plant. Gum-arabic (40% w: v) was used as a sticker, and allowed to dry to some extent for 15 min (Fred *et al.* 1932). Each pot received 8 to 10 seeds/pot for large seeded species (bean), 10-15 seeds/pot for moderate seed size species, and 20-40 seeds/pot for small seeded species.Uniform seedlings were Selected, and thinned 8-14 days after emergence. Large seeded varieties were thinned to two plants per pot; while smaller seededspecies were thinned to 6 per pot.Control pots for each crop were also involved.At 60 days after sowing, all plants were harvested.Roots were cleaned of soil particles.Number of root nodule, fresh weight, shoot length and dry weight were measured. Dry weight of plant materials was determined by drying samples at 70 ^{0}c

for 4 days. Plants were then grinded before estimation of total nitrogen.

(2) Total nitrogen

Total nitrogen content of plants was determined by micro-Kjeldahl method modified by (Piper, 1950). Blank estimations were also run and the results were calculated on even dry basis.

(3) Statistical analysis

Analysis of variance (ANOVA) was also performed on data of all plant parameters measured using the SAS software package (SAS User's Guide Statistics SAS institute Inc., Cary. NC, USA).

Table (1): Physical Characters of the used soil

Soil Type	**Percent (%)**
Clay (%)	40.4
Silt (%)	31
Fine sand (%)	26
Coarse sand (%)	2.6
Texture grade	Clay loam

Table (2): Chemical analysis of the used soil

Character	**Amount**
pH	8.2
Organic matter (%)	1.51
Available P(ppm)	18.4
$CaCO_3$ (%)	2.14
Total N (%)	0.14

RESULTS AND DISCUSSION

A total of 25 rhizobia were recovered from different growing sites (9 from faba bean, 7 from white clover, 3 from fenugreek, 4 from pea and 2 from lentil). Isolates were considered fast-growing rhizobia depending on: growth rate, colony morphology, Gram stain, Congo red absorption, acid/alkali production andare finally confirmed as rhizobia by the nodulation test.*Rhizobium* strains are rods, Gram-negative; colonies are circular, convex, semi-translucent or opaque, raised and mucilaginous (with high amount of mucus), usually 2-4mm in diameter within 3-5 days on YEMA media, commonly pleomorphic under adverse conditions (Holt *et al.* 1994 ;Kaur*et al*. 2012).The CR absorption test also indicated that none of the isolates absorbed CR in YEMA plates. This is a distinctive character of rhizobia with only few exceptions (Somasegaran and Hoben. 1994;Shetta*et al.* 2011).Production of mucous is in close agreement with Baoling*et al.*(2007).However, the colonies of all isolates obtained by Naz*et al*. 2009were almost nonmucilaginous and transparent.Colonies of our isolates differed in elevation(from low convex to high convex or semi-sphere), color (from watery to white)(Fig.1), transparency (from opaque to semi-translucent) and diameter (some reached 2mm after 5 days and others were 4-5 mm after the same period), as illustrated in Table (3).In this study, isolates from the same host plant were divided into groups after recording colony morphology of each. Hence, faba bean rhizobia were distributed into 4 groups, pea rhizobia were placed in 1 group, fenugreek rhizobia involved 2 groups,lentil rhizobia involved 1group and white clover rhizobia involved 1 group.

General microscopic view of the *Rhizobium* isolates showed them to be rod cells (Fig.2) and Gram-negative in nature (Singh *et al.* 2008;Kumari*et al.* 2010).

All isolateschanged the color of YEMA supplemented with BTB to yellow indicating that they are acid producers and hence possible to categorize them as fast-growers (i,e: *Rhizobium*).Acid production was observed by Shetta*et al.* (2011) andBaoling*et al.*(2007).

All*Rhizobium* isolates showed motility(Fig.3). This result is in line

withShahzad*et al.*(2012) andDubey*et al.* (2010).Despite that, very few researchers demonstrated incompatible results. Naz*et al*. 2009 reported that under the light microscope all the isolates were non-motile.

It was known that *Rhizobium* and *Bradyrhizobium* can't hydrolyze starch,casein and gelatin(Kuykendall *et al.* 2005).All our isolates of *Rhizobium* didn't produce amylase, casease and gelatinase, and all isolates gave no slime on starch medium except (2RH1). However, De Oliveira *et al.* (2007) observed that*Rhizobium* strains can utilize starch obtained from different sources. Kumari*et al*. 2010 also reported that 1 out of 5 isolates could use casein.All isolates of rhizobia collected by (Naz*et al*. 2009) were negative for gelatinase, while some fast- growing rhizobia isolated by Shetta*et al*. 2011, could hydrolysegelatin.

Bacterization of seeds with adhesive is the most suitable among the inoculation methods used and inoculation is responsible for good plant growth (Saha and Haque. 2005). *Rhizobium* isolates differed in their ability to enhance plant growth (No. of. nodules, shoot height, dry weightof whole plant, N %), with the highest results for 14RV,4(RV), RV, 3RLe, 2RHb, 1RH, 3RB, 4RT, 1RT, 4RT3 and 5RT(Table4).14Rv gave large nodules (Fig.4).Application of the rhizobial inoculant significantly increased the soybean yields in all mandate areas (about 75% of the farms)(Lesueur*et al.* 2012). However, the inoculation of soybean with rhizobial strains does not necessarily result in yield increase (Saeki*et al.* 2006).

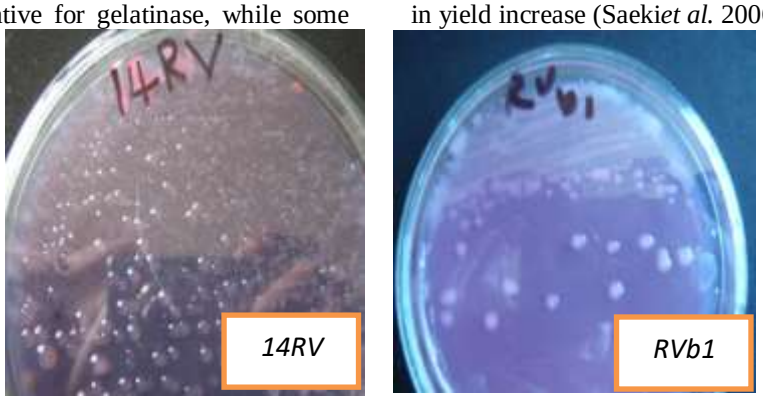

Fig (1): Cultures of *Rhizobium* on YEMA-CR medium after 6days

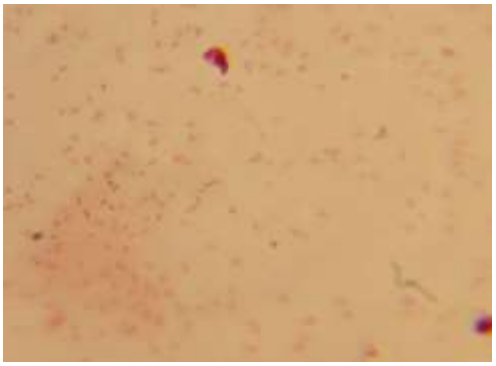

Fig (2)*:* Gram stained *Rhizobium* under light microscopy (100 x lenses) after 2-3days in YEMB

Table(3): Colony morphology and acid/alkali production of *Rhizobium*

Isolate	Elevation	Color	Transparency	Diameter(mm)		YMA-BTB
				4Days	5 Days	
Viciafaba(Faba bean)						
Group 1						
14RV	High convex	Very Pale White	translucent	1	2	y acid
Group 2						
8RV--	Low convex	White	Opaque	2.5	4	y acid
RVb1	Low convex	White	Opaque	2.5	4	y acid
Group 3						
11RV1	Low convex	Pale White	Semi translucent	1	2	y acid
6RV1	Low convex	Pale White	Semi translucent	1	2	y acid
Group 4						
3RV*	Low convex	White	Semi translucent	1	2	y acid
4RV*	Low convex	White	Semi translucent	1	2	y acid
1RV- -	Low convex	White	Semi translucent	1	1.5mm(6days)	y acid
4(RV)	Low convex	White	Semi translucent	1	1.5	Yacid
Pisumsativum L(pea)						
Group1						
2RBb*	Low convex	White	Opaque	2	3	y acid
3RBb	Low convex	White	Opaque	2	3	y acid
4RB\	Low convex	White	Opaque	3	4	y acid
3RBb*	Low convex	White	Opaque	2.5	4	Yacid
Trigonellafoenumgraecum(fenugreek)						
Group 1						
1RH	convex	Very Pale White	translucent	3.5	4	y
2RHb	convex	Very Pale White	translucent	3.5	4	y
Group 2						
2RH1	Low convex	Pale White	Semi translucent	1	2mm(6days)	y acid
Lens culinaris Medik. (Lentil)						
Group 1						
3RLe	Low convex	Pale White	Semi translucent	2	3	y acid
7RLe	Low convex	Pale White	Semi translucent	2	2.5	y acid
Trifoliumrepens (White clover)						
Group 1						
5RT	Low convex	White	Opaque	3	4.5	y acid
3RT\	Low convex	White	Opaque	2	5	y acid
2RT2	Low convex	White	Opaque	3	4	y acid
1RT*	Low convex	White	Opaque	3	4	Yacid
4RT	Low convex	White	Opaque	2	4	y acid
4RT3	Low convex	White	Opaque	2	4	y acid
6RT*	Low convex	White	Opaque	2	4	Yacid

Very Pale White =watery, High convex=semi-sphere. YEMA – BTB = YEMA with bromothymol blue.

y = yellow culture,y acid = yellow culture&the medium changed to yellow.

Table (4): Nodulation test of *Rhizobium* sp

Isolate	*No.of.nodules*	*Shoot height(cm)*	*Dry weight of whole plant (g)*	**N%**
Viciafaba(Faba bean)				
Control	0.00^{f}	23.33^{d}	1.33^{c}	2.18^{f}
RV	98.00^{b}	28.33^{abc}	2.47^{a}	3.08^{bc}
14RV	39.00^{e}	29.33^{ab}	2.03^{b}	3.49^{a}
8RV	57.00^{cde}	25.33^{cd}	1.82^{b}	2.31^{ef}
4(RV)	109.00^{a}	32.00^{a}	2.60^{a}	3.37^{ab}
6RV	79.00^{bc}	28.00^{bc}	1.82^{b}	2.31^{ef}
11RV	62.00^{cd}	28.66^{abc}	1.90^{b}	2.33^{ef}
3RV	65.00^{cd}	28.00^{bc}	2.01^{b}	2.96^{cd}
4RV	96.00^{ab}	30.66^{ab}	1.87^{b}	2.88^{cd}
1RV	50.00^{de}	27.33^{bc}	1.90^{b}	2.63^{de}
SE±	6.908931	1.1737878	0.11562391	0.10937702
Significance	**	**	**	**
Trigonellafoenumgraecum(fenugreek)				
Control	0.00^{c}	17.33^{b}	0.14^{c}	1.54^{c}
1RH	37.33^{a}	27.66^{a}	0.23^{a}	2.69^{a}
2RH1	24.66^{b}	26.33^{a}	0.18^{b}	2.07^{b}
2RHb	35.00^{a}	27.33^{a}	0.23^{a}	2.45^{a}
SE	2.9814240	1.3642255	0.00927961	0.11089284
Significance	**	**	**	**
Lens culinaris Medik. (Lentil)				
Control	0.00^{b}	12.16^{b}	0.17^{b}	2.72^{b}
7RLe	38.00^{a}	15.33^{a}	0.28^{a}	3.27^{b}
3RLe	40.33^{a}	17.33^{a}	0.28^{a}	3.90^{a}
SE±	2.2443344	0.8660254	0.01319371	0.16763055
Significance	**	*	**	**
Trifoliumrepens L (White clover)				
Control	0.00^{d}	13.66^{d}	0.06^{b}	2.16^{e}
3RT	18.33^{c}	20.00^{bc}	0.11^{a}	2.40^{de}
5RT	22.33^{bc}	22.33^{abc}	0.11^{a}	2.96^{cd}
2RT2	21.00^{bc}	21.00^{bc}	0.09^{ab}	2.56^{cde}
4RT	29.66^{a}	26.00^{a}	0.13^{a}	3.13^{bc}
1RT	25.33^{ab}	24.66^{ab}	0.12^{a}	3.83^{a}
4RT3	24.00^{abc}	22.66^{abc}	0.11^{a}	3.69^{ab}
6RT	20.33^{bc}	19.66^{c}	0.09^{ab}	2.63^{cde}
SE±	1.8219343	1.4385564	0.01307032	0.19485750
Significance	**	**	*	**
Pisumsativum L(pea)				
Control	0.00^{d}	23.66^{b}	0.85^{c}	1.86^{c}
2RB	28.33^{c}	32.33^{a}	1.77^{b}	2.05^{bc}
4RB	64.33^{a}	30.66^{a}	1.45^{b}	2.27^{b}
3RB*	37.00^{bc}	32.66^{a}	1.43^{b}	2.11^{bc}
3RB	44.00^{b}	37.33^{a}	2.3^{a}	2.84^{a}
SE±	4.5230521	2.0439613	0.14198591	0.12050818
Significance	**	**	**	**

Means with the same letter are not significantly different.

** = high significant (p<0.01),* = significant (p=0.01- 0.05).

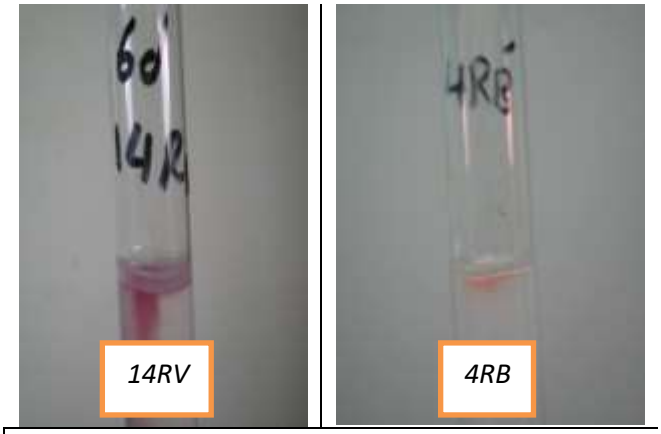

Fig (3): Motility test of *Rhizobium* after 6days.

(1) (2) (3)

Fig (4): Nodulation test of Rhizobium; (1, 2): *Viciafaba* inoculated with RVb1 and 14RV, respectively. (3): control plant. 14 RV gave Larger nodules.

REFERENCES

Aneja, K.R. (2003). Experiments in microbiology plant pathology and biotechnology, 4th. ed, New Age International Publishers, New Delhi, India.

Baoling, H.; ChengQun, L; Bo, W. and LiQin, F. (2007).A rhizobia strain isolated from root nodule of gymnosperm *Podocarpusmacrophyllus. Sci.Chin. Ser C-Life Sci.*, 50: 1- 6.

Chen, W.M.; Laevens, S.; Lee, T.M.; Coenye, T.; de Vos, P.; Mergeny, M. and Vandamme, P. (2001).*Ralstoniataiwanensis*sp. nov.isolated from root nodules of *Mimosa* species and sputum of a cystic fibrosis patient, Int .J.Syst. Evol.Microbiol, 51: 1729-1735.

De Oliveira, A.N.; De Oliveira, L.A.; Andrade, J.S. and Chagas, J.A.F. (2007). Rhizobia amylase production using various starchy substances as

carbon substrates, Braz. J. Microbiol, 38: 208-216.

Dubey, R. C.; Maheshwari, D. K.; Kumar, H. and Choure, K. (2010).Assessment of diversity and plant growth promotingattributes of rhizobia isolated from *Cajanuscajan*L.African Journal of Biotechnology. 9(50). 8619-8629.

Fauvert, M. andJ.Michiels.(2008)."Rhizobial secreted proteins as determinants of host specificity in the *Rhizobium*-legume symbiosis." FEMS Microbiology Lett. 285: 1-9.

Fred, E.B ; Baldwin, I .L. and McCoy, E. (1932) , Root nodule bacteria and leguminous plants, Studies Science University of Wisconsin 5 Madison.

Holt, J.G.; Kreig, N.R.; Sneath,P.H.A.; Staley, J.T. and Williams, S.T. (1994) . Bergey's Manual of Determinative Bacteriology, 9th ed. p. 40-169, Williams and Wilkins. (ed.). Baltimore, USA,.

Kaur, H.; Sharma, P.; Kaur, N. and Gill, B. S. (2012).Phenotypic and biochemical characterization of *Bradyrhizobium* and *Ensifer* spp. isolated from soybean rhizosphere, Bioscience Discovery, 3(1):40-46.

Kumari, B. S.; Ram, M. R. and Mallaiah, K. V. (2010).Studies on nodulation, biochemical analysis

and protein profiles of *Rhizobium* isolated from *Indigofera* species, Malaysian Journal of Microbiology, 6(2) 133-139.

Kuykendall, L.D.; Young, J.M.; Martı´nez-Romero,E.; Kerr,A. and Sawada, H. (2005). Order Rhizobiales (new) family Rhizobiaceae genus *Rhizobium*, p 324–340, In: Brenner,D.J.; Krieg,N.R; Staley,J.T. and Garrity,G.M. (ed.), The alpha-, beta-, delta-, and Epsilonproteobacteria, the Proteobacteria, Part C, Bergey's manual of systematic bacteriology, vol 2, 2nd edn. Springer, New York.

Larone, D. H. (1993). Medically important fungi: a guide to identification, 2nd ed., p. 193-211. American Society for Microbiology, Washington, D.C.

Lesueur, D.; Atieno, M. ;Mathu, S. and Herrmann, L. (2012). Importance of rhizobia in Agriculture: potential of the commercial inoculants and native strains for improving legume yields in different land-use systems,Geophysical Research Abstracts,Vol. 14, EGU2012-632, 2012.

MacFaddin, J. (1972). Biochemical tests for the identification of medical bacteria, Williams and Wilkins Company, Baltimore, MD.

Medeot, D.B.; Paulucci, N.S.; Albornoz, A.I.; Fumero, M.V.; Bueno, M.A.; Garcia, M.B.; Woelke, M.R.; Okon,Y. and Dardanelli, M.S. (2010). Plant Growth Promoting Rhizobacteria Improving the Legume–Rhizobia Symbiosis, p.467, Khan .M.S, Zaidi.A.andMusarrat.J. (ed.), Microbes for Legume Improvement , Springer-Verlag/Wien.Germany.

Naz, I.;Bano, A.andTamoor-UL-Hassan. (2009). Morphological, biochemical and molecular characterization of rhizobia from halophytes of Khewra salt range and Attock, Pak. J. Bot., 41(6): 3159-3168.

Piper, C.S.(1950). Soil and plant analysis, Inter Science Publishers, New York.

Saeki, Y.; Aimi, N.; Tsukamoto, S.; Yamakawa, T.; Nagatomo, Y. and Akao, S. (2006). Diversity and geographical distribution of indigenous soybean-nodulatingbradyrhizobia in Japan.

Soil Science and Plant Nutrition, 52: 418–426.

Saha, A.K. and Haque, M.F. (2005).effect of inoculation with *Rhizobium* on nodulation and growth of bean, *Dolichos lablab*. J. Life Earth Science, 1(1):71-74.

Sawada, H.; Kuykendall, L.D. and Young, J.M. (2003).Changing concepts in the systematics of bacterial nitrogen-fixing legume symbionts.J Gen ApplMicrobiol. 49:155–179.

Shahzad, F. ;Shafee, M.; Abbas, F.; Babar, S.; Tariq, M. M. and Ahmad, Z. (2012). Isolation and biochemical characterization of *Rhizobium melilotif*rom root nodules of alfalfa (*Medico sativa)*.The Journal of Animal & Plant Sciences, 22(2): 522-524.

Shetta, N.D.; Al-Shaharani, T.S. and Abdel-Aal, M. (2011). Identification and characterization of *Rhizobium* associated with woody legume trees grown under Saudi Arabia condition, American-Eurasian J. Agric. & Environ. Sci., 10 (3): 410-418.

Singh, B.; Kaurand, R. and Singh, K. (2008).Characterization of *Rhizobium* strain isolated from the roots of *Trigonellafoenumgraecum (*fenugreek).African J. Biotechnol., 7(20): 3671-3676.

Somasegaran, P. and Hoben, H.J. (1994).Handbook for Rhizobia methods in Legume-*Rhizobium* Technology, springer Verlag, New York, USA.

Sylvia, D.M.; Fuhrmann, J.J.; Hartel, P.G. and Zuberer, D.A. (2005). Principles and applications of soil microbiology, 2nd.ed, Table 16-2, p. 409.

Talaro, K.P. (2009). Foundations in microbiology, McGraw-Hill companies.Inc. New York. 7thedn.P.794-796.

Tortora, G.J; Funke,B.R. and Case, C.L.(1982). "Microbiology: an introduction",The Benjamin/Cummings publishing company,Inc.California.p.668-672.

Valverde, A.; Vela´zquez, E.; Ferna´ndez-Santos, F.; Vizcaı´no, N.; Rivas, R.; Mateos, P.F.; Martı´nez-Molina, E.; Igual, J.M. and Willems, A. (2005). *Phyllobacteriumtrifolii* sp. nov., nodulating*Trifolium* and *Lupinus* in Spanish soils. Int J SystEvolMicrobiol. 55:1985–1989.

Valverde, A.; Vela´zquez, E.; Gutirrez, C.; Cervantes, E.; Ventosa, A. and Igual, J.M. (2003).*Herbaspirillumlusitanum* sp. nov., a novel nitrogen-fixing bacterium associated with root nodules of *Phaseolus vulgaris*. Int J SystEvolMicrobiol 53:1979–1983.

Vela´zquez, E.; Garcı´a-Fraile, P.; Rivas, R.; Martı´nez-Molina, E. and Ramı´rez-Bahena, M. (2010). Bacteria Involved in Nitrogen-Fixing Legume Symbiosis: Current Taxonomic Perspective, p1- 6, In:Khan.M.S. et al. (ed.) , Microbes for Legume Improvement.Springer-Verlag/Wien.Gerrmany.

Vincent, J.M. (1970). A manual for the practical study of root-nodule bacteria, IBP handbook 15.Blackwell Scientific Publications, Oxford.

Yadav, A.S. (2008). Resistance to sodium azide and its effect on symbiotic effectivity of rhizobial strains: a review. *Natnl. J. Pl. Improv*., 10(2): 75.

Zahran,H.H. (2010). Legumes–Microbes Interactions Under Stressed Environments, Khan .M.S, Zaidi.A.andMusarrat.J. (ed.).

Microbes for legume improvement, Springer-Verlag/Wien, p.355.

Zurdo-Pin˜eiro, J.L.; Rivas, R.; Trujillo, M.E.; Vizcaino, N.; Carrasco, J.A.; Chamber, M.; Palomares, A.; Mateos, P.F.; Martı´nez-Molina, E. and Vela´squez, E. (2007). *Ochrobactrumcytisi* sp. nov., isolated from nodules of *Cytisusscoparius* in Spain. Int J SystEvolMicrobiol. 57:784–788.

الملخصالعربي

دراسة الصفات العامة وكفاءة الرايزوبيوم المعزول من بعض النباتات البقولية

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تم عزل 25 عزلة للرايزوبيوم من أماكن مختلفة بمحافظة المنيا، وتم تعريفهم كريزوبيوم سريع النمو بناء على: معدل النمو، مورفولوجى المستعمرة، صبغة جرام، امتصاص صبغة الكونجو الحمراء، انتاج حمض أو قاعدة، القدرة على الحركة، اختبار تحلل الكازين، تحلل الجيلاتين وتحلل النشا، وأخيرا تم التأكد من كونها رايزوبيا من خلال قدرتها على تكوين العقد. كانت كل العزلات مخاطية، لها مستعمرات دائرية ولها القدرة على الحركة ولم تمتص صبغة الكونجو الحمراء. واختلفت من حيث كونها مرتفعة الى عالية التحدب ومن بيضاء الى مائية اللون ومن معتمة الى شفافة. كانت العزلات سالبة جرام وأعطت أحماض من المانيتول. لم تستطع أي منها أن تحلل النشا أو الكازين أو الجيلاتين. جميعها استطاع تكوين عقدعلى النباتاتالتي عزلت منها ولكن بكفاءات مختلفة.