

Plasmid profile of two isolates of *Sinorhizobium meliloti* isolated from different soil areas in Basrah/ Iraq

Ghosooun F. Al-Kanaany, Hisham F. Al-Yassri and Adnan A. Al-Mousawi
Department of Biology, College of Science, University of Basrah, Iraq

Abstract. Genetic diversity of two isolates of *Sinorhizobium meliloti* grown in different soil samples were investigated. The results showed that two isolates of *S. meliloti* were different in their plasmid profile. One of these isolates was containing one large megaplasmid with molecular weight (1.6Mb) and the other isolate was containing two large megaplasmid with molecular weight (1.6Mb and 1.3Mb). pSymA and pSymB in *S. meliloti* are involved in the formation and functioning of nitrogen-fixing root nodules. Genes located on pSymA are necessary for nodulation and nitrogen fixation, while those located on pSymB are involved in exopolysaccharide synthesis and uptake of various nutrients.

Key words: *Sinorhizobium meliloti* , Plasmid profile, Genetic diversity.

Introduction

The symbiosis between legumes and N₂-fixing bacteria (rhizobia) is of huge agronomic benefit, allowing many crops to be grown without N₂ fertilizer. It is a sophisticated example of coupled development between bacteria and higher plants, culminating in the organogenesis of root nodules (7). There have been many genetic analysis of rhizobia, notably of *Sinorhizobium meliloti* (the symbiont of alfalfa), *Bradyrhizobium japonicum* (soybean), and *Rhizobium leguminosarum*, which has Biovars that nodulate peas and broad beans (Biovar *viciae*), clovers (Biovar *trifolii*), or kidney beans (Biovar *phaseoli*).

Genomes of the plant-associated bacteria are still larger. The genome of *Agrobacterium tumefaciens* is about 5.6 Mb, with one circular and one linear chromosome, plus two native plasmids (10, 28). To date, three rhizobial genomes have been sequenced. *S. meliloti* 1021 has a 3.5 Mb chromosome plus two megaplasmids, namely pSymA

and pSymB, with the former having genes for nodulation (*nod*) and symbiotic N₂ fixation (*nif* and *fix*)(8). In contrast, the symbiosis genes of *Mesorhizobium loti* MAFF303099 (which nodulates *Lotus*) and of *B. japonicum* USDA110 are on chromosomal 'symbiosis islands', with the chromosome of the latter (9.1 Mb) being among the largest yet known in bacteria(14, 15). *nif* and *fix* genes are present on chromosome in *Rhizobium loti*, *Bradyrhizobium* spp. And *Azorhizobium* spp. These genes are located on symbiotic plasmid in *S. meliloti*, *R. leguminosarum* and *Rhizobium* spp. NGR234 (25) *Rhizobium leguminosarum* has yet another genomic architecture: one circular chromosome and several large plasmids, the plasmid profile varying markedly among isolates in terms of sizes, numbers, and incompatibility groups (18, 24). The subject of the present study, *R. leguminosarum* biovar *viciae* (Rlv) strain 3841 (a spontaneous streptomycin-resistant mutant of field isolate 300 (9, 13), has six large plasmids; pRL10 is the pSym (symbiosis plasmid). It is very

important to study the native population of rhizobial bacteria in Iraq, where it is supposed to be the origin of the host plant for *Sinorhizobium* genus. On the other hand it has been documented that the evolution of symbiotic rhizobacteria may be influenced by environmental factors (26).

The aim of this study was to investigate the native population diversity of indigenous *Sinorhizobium meliloti* bacteria isolated from two different areas of Basrah /Iraq soils (A: Shatt Alarab, B: Qarma city) by using Plasmid profile .

Materials and Methods

Bacterial isolates

Two isolates of *Sinorhizobium. meliloti* were isolated from nodules of alfalfa (*Medicago. sativa*) plants from different geographical sites of Basrah /Iraq according to Vincent (27) method. These isolates were identified by Al-Kanany (2). Pure isolate was grown on the nutrient agar for other use.

Genomic DNA extraction

S. meliloti was grown in nutrient broth at 28 °C for 24 hr. and genomic DNA was isolated using the Wizard genomic DNA purification kit Serial No. 214567 (Promega, Madison, WI, U.S.A.).

Plasmid profiles

The plasmid was isolated using the QIAgene miniprep kit Serial NO. 27104 U.S.A. .

Results and Discussion

Previous studies had not shown the genome of *Sinorhizobium meliloti* symbiont with alfalfa (*M. sativa*) in Iraq. In this study we isolated the total DNA and plasmid profile from *S. meliloti* for the first time in Iraq. The two megaplasmsids pSymA and pSymB of *S. meliloti* carry many genes that are involved in uptake and assimilation of various organic and inorganic nutrients

such as dicarboxylate acids, sugars, aromatic compounds, osmoprotectants (betaines, ectoines), iron, and inorganic phosphate.

Plasmid profile:

The results demonstrated that two isolates of *Sinorhizobium meliloti* showed differences in their plasmid profile. One isolate carried one large Megaplasmsid with molecular weight (1.6 Mb) and the other isolate carried two.

This indicated that Rhizobial plasmid size varied among rhizobial bacteria isolates (12, 20, 30).

This difference between two isolates in plasmid profile showed that genetic diversity may be affected by environmental conditions such as biological barrier of gene exchange, geographical isolation, soil types and genotype of the host plant (6, 16, 17, 21). Our results also indicated that genetic variations between the studied isolates based on differentiation of soils (A: Shatt Al-arab and B: Qarma city). These results were agreed with the results of Abdel-Aziz (1).

The large native population of *S. meliloti* presents in Basrah/ Iraq soils appeared to be different in tolerant to ecological factors of salt and pH stress (2).

It is very important to study plasmid of *S. meliloti* because many of their genes, which are important in symbiosis, are plasmid borne. In *S. meliloti*, genes required for nodulation and nitrogenase activity have been mapped on the mega plasmid (pSymA) (5, 19). The second megaplasmsid, pSymB, carries genes involved in exopolysaccharide synthesis, thiamine synthesis and dicarboxylate transport (29) and the other studies showed that the *nod* and *nif* genes is located on the large plasmid (3, 22).

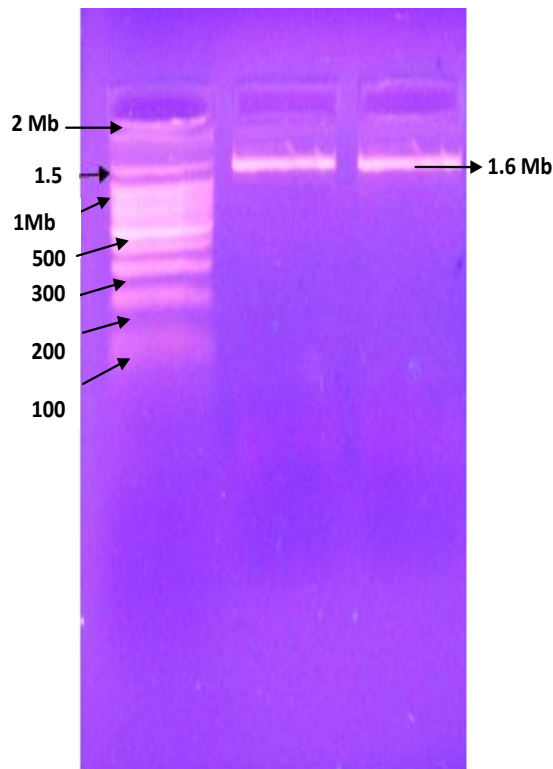


Fig. (1):: Gel electrophoresis profile of *Sinorhizobium meliloti* carrying one large mega plasmid (1.6 Mb) Agarose gel (0.8%).

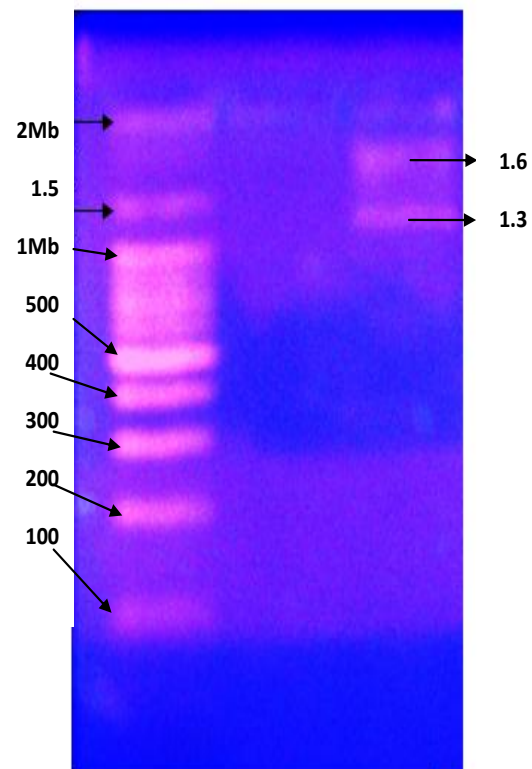


Fig. (2): Gel electrophoresis profile of *Sinorhizobium meliloti* carrying two Mega plasmid (1.6Mb, 1.3 Mb) Agarose gel (0.8%).

Our results are agreed in the size of plasmids with the results of Galibert *et al* (8) who demonstrated that *S. meliloti* contains two megaplasmid with molecular weight (1.65 Mb and 1.35 Mb) and Oresink *et al* (23) showed that *S. meliloti* typically contains two megaplasמידs of approximately 1.400 and 1.600 kb, Other studies showed that *S. meliloti* contains one megaplasמיד with molecular weight of about (1500 kb) or even larger (4). two megaplasמיד of about (1400 kb) (pSymA or megaplasמיד 1) and (1700 kb (pSym B or megaplasמיד 2)) are present in *S. meliloti* (11).

In conclusion, genetic diversity of *Sinorhizobium meliloti* bacteria isolated from Basrah/ Iraq was different in their Plasmid profile and showed that soil properties effect on plasmid diversity.

References

- 1- Abdel-Aziz, R.A.; Al-Barakah, F.N. and Al-Asmary H.M. (2008). Genetic identification and symbiotic efficiency of *Sinorhizobium meliloti* indigenous to Saudi Arabian soils. African Journal of Biotechnology. 16: 2803-2809.
- 2- Al-Kanany, G.F.R. (2008). Isolation of *Sinorhizobium meliloti* symbiont of *Medicago sativa* L. with induction of mutation resistant to salinity by using ultra violet .Msc . Thesis, College of Science, Basrah Unvercity, 117 pp.
- 3- Barloy-Hubler, F. D.; Capela, M.J.; Barnett, S.; Kalman, N.A.; Federspiel, S.R.; and Gilbert, F.(2000). High-Resolution Physical Map of the *Sinorhizobium meliloti* 1021 p Syma Mega. J. Bacteriol., 182: 1185-9

- 4- Burkardt, B. and Burkardt, H.J. (1984). Visualization and exact molecular weight determination of a *Rhizobium meliloti* megaplasmid. *J. Mol. Biol.*, 175: 213-218.
- 5- Batut, J.; Terzaghi, B.; Gherardi, M.; Huguet, M.; Terzaghi, E.; Garnerone, A.M.; Boistard, P. and Huguet, T. (1985). Localization of symbiotic fix region on *Rhizobium meliloti* pSym megaplasmid more than 200 kilobases from the nod –nif region. *Mol. Gen. Genet.* 199:232-239 .
- 6- Coutinho, H.L.; Oliveria, C.; Lovato, V.M.A.; Maia A.H.N. and Manfio, G.P. (1999). Evaluation of the diversity of rhizobia in Brazilian agricultural soils cultivated with soybeans. *Appl. Soil Ecol.*, 13: 159–67
- 7- Gage, D.J. (2004). Infection and invasion of roots by symbiotic, nitrogen-fixing rhizobia during nodulation of temperate legumes. *Microbiol Mol Biol Rev.*, 68: 280–300.
- 8- Galibert, F.; Finan, T.M.; Long, S.R.; Puhler, A.; Abola, P.; Ampe, F.; Barloy-Hubler, F.; Barnett, M.J.; Becker, A.; Boistard, P. (2001). The composite genome of the legume symbiont *Sinorhizobium meliloti*. *Science.*, 293: 668–672.
- 9- Glenn, A.R.; Poole, P.S.; Hudman, J.F. (1980). Succinate uptake by free-living and bacteroid forms of *Rhizobium leguminosarum*. *J Gen Microbiol.*;119:267–271.
- 10- Goodner, B., Hinkle, G. Gattung, S. Miller, N. Blanchard M, Quorllo B, Goldman BS, Cao Y.W, Askenazi M, Halling C,. (2001). Genome sequence of the plant pathogen and biotechnology agent *Agrobacterium tumefaciens* C58. *Science.*, 294: 2323–2328.
- 11- Hynes, M.F.; Simon, R.; Muller, P.; Niehaus, K.; Labes, M. and Puhler, A. (1986). The two megaplasms of *Rhizobium meliloti* are involved in the effective nodulation of alfalfa. *Mol. Gen. Genet.* 202 :356-362 .
- 12- Jebara, M.; Mhamdi, R.; Aouani, M.E.; Ghir, R. and Mars, M. (2001). Genetic diversity of *Sinorhizobium* populations recovered from different Medicago varieties cultivated in Tunisian soils. *Canadian J. Microbiol.*, 47: 139–47.
- 13- Johnston, A.W.B.; Beringer, J.E. (1975). Identification of *Rhizobium* strains in pea root nodules using genetic markers. *J Gen Microbiol.*;87:343–350.
- 14- Kaneko, T.; Nakamura, Y.; Sato, S.; Minamisawa, K.; Uchiumi, T.; Sasamoto, S.; Watanabe, A.; Idesawa, K.; Iriguchi, M.; Kawashima, K. (2002). Complete genomic sequence of nitrogen-fixing symbiotic bacterium *Bradyrhizobium japonicum* USDA110. *DNA Res.*, 9: 189–197.
- 15- Kaneko, T.; Nakamura, Y.; Sato, S.; Asamizu, E.; Kato, T.; Sasamoto, S.; Watanabe, A.; Idesawa, K.; Ishikawa, A.; Kawashima, K. (2000). Complete genome structure of the nitrogen-fixing symbiotic bacterium *Mesorhizobium loti*. *DNA Res.*; 7:331–338.
- 16- Krasovsky, V. N. and Stotzky, G. (1987). Conjugation and genetic recombination in *Escherichia coli* in sterile and non-sterile soil. *Soil Biol. Biochem.*, 19: 631-8.
- 17- Lakzian, A. and Bromfield, E. (2004). The effect of trap host plants on the population diversity of *Bradyrhizobium japonicum*. *Iranian J. Biotechnol.*, 2: 90–6
- 18- Laguerre, G.; Mazurier, S.I.; Amarger, N. (1992). Plasmid profiles and restriction fragment length polymorphism of *Rhizobium leguminosarum* bv *viciae* in field populations. *FEMS Microbiol Ecol.*;101:17–26.
- 19- Lamb, J.W.; Hombrecher, G. and Johnston, A.W.B. (1982). Plasmid –

- determined nodulation and nitrogen – fixation abilities in *Rhizobium phaseoli* Mol. Gen. Genet. 186: 449-452.
- 20- Lakzian, A. (1998). Diversity and metal tolerance of *Rhizobium leguminosarum* bv. *viciae* in soils contaminated with heavy metals. Ph.D. Thesis, University of London.
- 21- Mendes, I.C. and Bottomley, P.J. (1998). Distribution of a population of *Rhizobium leguminosarum* Bv. *trifoli* among different size classes of soil aggregates. Appl. Environ. Microbiol., 64: 970–5
- 22- Melanie, J.B.; Fisher, R.F.; Jones, T.; Komp, C.; Abola, A.P.; Barloy-Hubler, F.; Bowser, L.; Capela, D.; Gilbert, F.; Gouzy, J.; Gurjal, M.; Hong, A. L.; Huizar, R.W.; Hyman, D.; Kahn, M.L.; Kahn, S.; Kalman, D.H.; Keating, C.; Palm, M.; Peck, C.; Surzycki, R.D.H.; Wells, K. Yeh, R.W.; Davis, N.A.; Federspiel and Long, S.R. (2001). Nucleotide sequence and predicted functions of the entire *Sinorhizobium meliloti* pSymA megaplasmid. PNAS, 98: 9883–8.
- 23- Oresnik, I.J.; Liu, S.L.; Yost, C.K. and Hynes, M.F. (2000). Megaplasmid pRme 2011a of *Sinorhizobium meliloti* is not required for viability. J. Bacteriology, 182: 3582-3586.
- 24- Palmer, K.M.; Turner, S.L.; Young, J.P.W. (2000). Sequence diversity of the plasmid replication gene *repC* in the *Rhizobiaceae*. Plasmid., 44: 209–219.
- 25- Rosenberg, C.; Boistard, P.; Denarie, J. and Casse- Delbart, F. (1981). Genes controlling early and late functions in symbiosis are located on a megaplasmid in *Rhizobium meliloti* .Mol.Gen .Genet .184:326-333 .
- 26- Souza, V.; Equiarte, L.; Avila, G.; Cappello, R.; Gallardo, C.; Montoya, J. and Piñero, D. (1994). Genetic Structure of *Rhizobium etli* biovar *phaseoli* Associated with Wild and Cultivated Bean Plants (*Phaseolus vulgaris* & *Phaseolus coccineus*) in Morelos, Mexico, Appl. Environ. Microbiol., 60: 1260–8.
- 27- Vincent, J. M. (1982) . Nitrogen fixation in legumes. Academic Press Sydney, New York, London.
- 28- Wood, D.W.; Setubal, J.C.; Kaul, R.; Monks, D.E.; Kitajima, J.P.; Okura, V.K.; Zhou, Y.; Chen, L.; Wood, G.E. and Almeida, N.F. (2001). The genome of the natural genetic engineer *Agrobacterium tumefaciens* C58. Science., 294: 2317–2323.
- 29- Watson, R.J.; Chan, Y.K.; Wheatcroft, R.; Yang, A-F. and Han, S. (1988). *Rhizobium meliloti* genes required for C4 –dicarboxylate transport and symbiotic nitrogen fixation are located on a megaplasmid .J. Bacteriol. 170: 927-934
- 30- Zou, X.; Feng, X.L.; Chen, W.X. and Li, F.D. (1998). Biological behavior of plasmid in *Rhizobium* sp. strain S25 from *Tephrosia candida*. Plasmid., 40: 158–63.

المحتوى البلازميدي لجرثومة *Sinorhizobium meliloti* المعزولة من تربة منطقتين مختلفتين في البصرة/ العراق

غصون فاضل الكنعاني، هشام فياض الياسري وعدنان عبدالله الموسوي

قسم علوم الحياة - كلية العلوم، جامعة البصرة، البصرة، العراق

المستخلص. ظهرت إختلافات في التركيب الوراثي لجرثومة *Sinorhizobium meliloti* المعزولة من تربة منطقتين مختلفتين من محافظة البصرة، اذ لوحظ وجود إختلاف في المحتوى البلازميدي لعزلتين من جرثومة *S. meliloti*. أشارت النتائج إلى إحتواء إحدى العزلتين على بلازميد واحد بوزن جزيئي (1,6) ميكابايت بينما اظهرت العزلة الاخرى احتواءها على بلازميدين بوزن جزيئي (1,3 و 1,6) ميكابايت. إن كل من البلازميدين (pSymA, pSymB) ضروريين في عملية تثبيت النتروجين حيث أن معظم الجينات المسؤولة عن عملية تكوين العقد وتثبيت النتروجين يكون موقعها على البلازميد (pSymA) بينما الجينات المسؤولة عن صنع السكريات المتعددة التي تدخل في تركيب الخلية الجرثومية و المواد المغذية المتنوعة التي يكون موقعها على البلازميد (pSymB). يستدل من هذه النتائج وجود إختلافات وراثية بين العزلات الجرثومية المعزولة من مناطق مختلفة.