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

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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 N_2-fixing bacteria (rhizobia) is of huge agronomic benefit, allowing many crops to be grown without N_2 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 N_2 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 asymbiotic 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

Tow 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 ċ 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 megaplasmids 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 Megaplasmid 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 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 megaplasmid, 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).

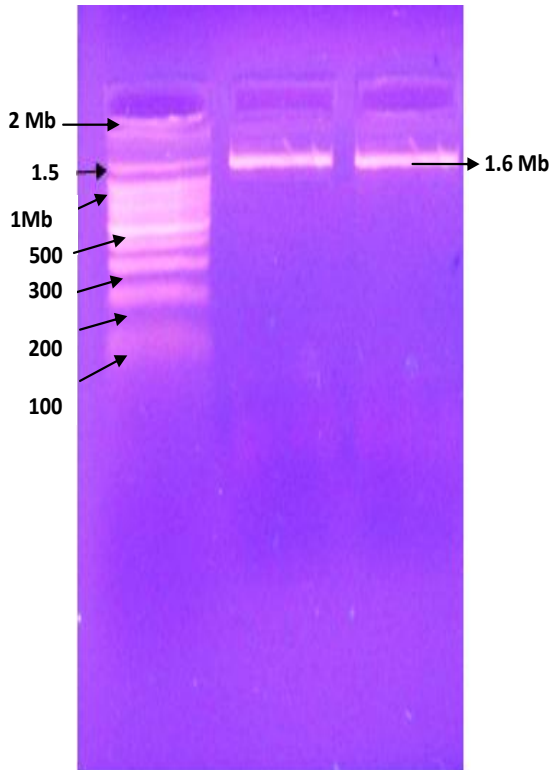

Fig. (1):: Gel electrophoresis profile of *Sinorhizobium meliloti* carrying one large mega plasmid (1.6 Mb) Agarose gel (0.8%).

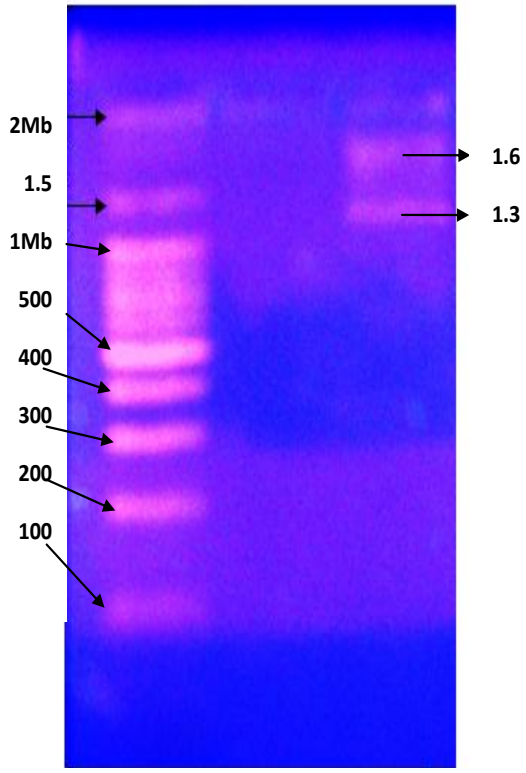

Fig. (2): Gel electrophoresis profile of *Sinorhizobium meliloti* carrying two Mega plasmid (1.6Mb, 1.3 Mb) Agarose gel (0.8%).

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 tow megaplasmids of approximately 1.400 and 1.600 kb, Other studies showed that *S. meliloti* contains one megaplasmid with molecular weight of about (1500 kb) or even larger (4). two megaplasmid of about (1400 kb) (pSymA or megaplasmid 1) and (1700 kb (pSym B or megaplasmid 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.

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

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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 و1,6) ميكابايت. إن كل من البلازميدين (pSymA,pSymB) ضرورين في عملية تثبيت النتروجين حيث أن معظم الجينات المسؤولة عن عملية تكوين العقد وتثبيت النتروجين يكون موقعها على البلازمبد (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.