Basrah J. Agric. Sci. 2012, 25(2): 27-33

Comparison of plasmid profile for three isolates of *Rhizobium leguminosarum* isolated from *Vicia faba* L. plant from different environmental soils of Iraq

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Abstract. The study observed the existence of different in size and number of plasmids for three *Rhizobium leguminosarum* isolates selected from different locations of Iraq isolated from root nodules of *Vicia faba* L. .These rhizobia were compared by plasmid profile .The results had indicated that one isolates(A: Nasryia) was containing four plasmids with molecular weight approximately 350 - 1000 kb and other isolate (B: Samawa) was carrying one plasmid with molecular weight approximately 700 kb and third isolate (C: Meisan) was containing two plasmids with molecular weight approximately 700 kb and 1000 kb. that mean there were differences in the plasmid diversity between three isolates obtained from different soils environments due to genetic variation that appeared among the studied isolates.

Key words: *Rhizobium leguminosarum,* Genetic variation, Plasmid.

Introduction

Rhizobium leguminosarum bv. viciae is an alphaproteobacterial (1) that can enter nitrogen-fixing symbioses (root nodules) with several legumes such as *Pisum*, *Vicia*, and *Lens* spp (2). The root nodule bacteria collectively known as the rhizobia have been studied intensively because of their economic importance as symbiotic nitrogen fixers, and inherent interest of biology of their complex interaction with plants. They are also interest because of the variety of their genome organizations and the complexity of their genomes (3). *Rhizobium leguminosarum* has yet another genomic architecture: One circular chromosome and several large plasmids (4,5). Most of the genes required for nodule formation (*nod*) and nitrogen fixation (*nif* and *fix*) are carried on a plasmid that is traditionally called the symbiotic plasmid or pSym (6,7). Plasmids are important genetic components for the divergence and adaptation of microbial populations because they contribute to genomic plasticity. Although plasmid profiles in rhizobia can be considered as a comparatively stable character, strains can lose some of their traits due to loss or partial deletion of a plasmid (8,9). *R. leguminosarum* is considered to be an obligate aerobe, and most of the genes in central metabolism are consistent with this, for example, the genome of Rlv3841 contains all of the genes for a functional TCA cycle on the chromosome are actually three candidate genes for citrate synthase (RL2508, RL2509, and RL2234) on the chromosome of Rlv3841. *R. tropici* has two citrate synthase genes, one of them, namely *pcsA*, is present on its pSym and affects nodulating ability and Fe uptake (10).The genome of Rlv3841 contains genes for isocitrate lyase (RL0761) and malate synthase (RL0054), which would allow a gloxylate cycle to operate, although strain 3841 does not grow on acetate. There are six genes whose product closely resemble succinate semialdehyde dehydrogenases (pRL100134, pRL100252, pRL120044, pRL120603, pRL120628, and RL0101), which could feed succinate semialdehyde directly into the TCA cycle. Two of them (pRL100134 and pRL100252) are on the symbiosis plasmid, and RL0101 is the characterized *gabD* gene (11). Succinate semialdehyde is the keto acid released from 4-aminobutyric acid, an amino acid that is present at high levels in pea nodules and is a possible candidate for amino acid cycling in bacteroids. The importance of this is that amino acid cycling has been proposed to be essential for productive N_2 fixation in pea nodules (12). A *Rhizobium leguminosarum* plasmid which determines hydrogen uptake ability and symbiotic functions in pea (13). Plasmids confer awide variety of traits on the bacteria that harbar them and are able to promote exchange of genetic information across taxonomic boundaries, they have undoubtedly figured heavily in bacterial evolution most of the genetic information of a bacterium is contained in a large circular DNA molecule, termed the chromosome and the rest resides in variable number of accessory genetic elements comprised of plasmids, bacterophage and transposable elements (14).

Bacteria with large complex genomes are abundant and important in nature. The aim of this study was to compare plasmid profile between three isolates of *Rhizobium leguminosarum* bv. *vicia* associated with *Vicia faba* L. plant isolated from different environmental areas of Iraq.

MATERIALS AND METHODS

Bacterial isolates

Three isolates of *Rhizobium leguminosarum* were isolated from nodules of *Vicia faba* L. plants from different geographical rigions of Iraq according to Vincent method (15). These isolates were obtained from (A: Nasryia, B: Samawa , C: Meisan).

Genomic DNA extraction

R. leguminosarum was grown in nutrient broth at 28 ċ for 24 hr and genomic DNA was extracted using the wizard genomic DNA purification kit, serial No. 214567. (Promega, Madison, WI, USA).

Plasmid extraction

The plasmid was extracted using the Qia gene miniprep kit serial NO. 27104. USA.

Results and Discussion

In this study we isolated the genome and plasmid profile from *Rhizobium leguminosarum* for the first time in Iraq because previous studies had not shown to genome of *R. leguminosarum* symbiont with *Vitia faba* L..

The results demonstrated that the three isolates of *R. leguminosarum* differ in their plasmid profile. One of them was (A: Nasryia) contained four plasmids, ranging with molecular weight 350 kb to approximately 1000 kb, While the (B) isolates that isolated from Samawa carried one plasmid with molecular weight approximately 700kb, and the other isolate was obtained from Meisan (C isolate) contained two plasmids with molecular weight approximately 700kb and 1000 kb fig(1). These differences among three isolates in plasmid profile has shown that genetic diversity affected by environmental conditions such as biological barrier of gene exchange, geographical isolation, soil types and genotype of the host plant (16, 17) .

Other studies found that *R. leguminosarum* strain Rlv3841 had six plasmids, but other natural *R.*

leguminosarum strains had from two to six plasmids of various sizes (4,18) .The pSym of Rlv3841 is pRL10 (488 kb) (5). but other pSyms differ in size (19). *Rhizobium leguminosarum* strain VF39. contains six plasmids (20) **.** All strains of *R. leguminosarum* had several large plasmids, for example *R leguminosarum* biovar viciae strain 3841 has a circular chromosome and 6 circular plasmids, ranging in size from approximately 140 to 870 kb. one of these plasmids was pRL10 carries the genes for nodulation and nitrogen fixation but the numbers and sizes of plasmids vary among strains.

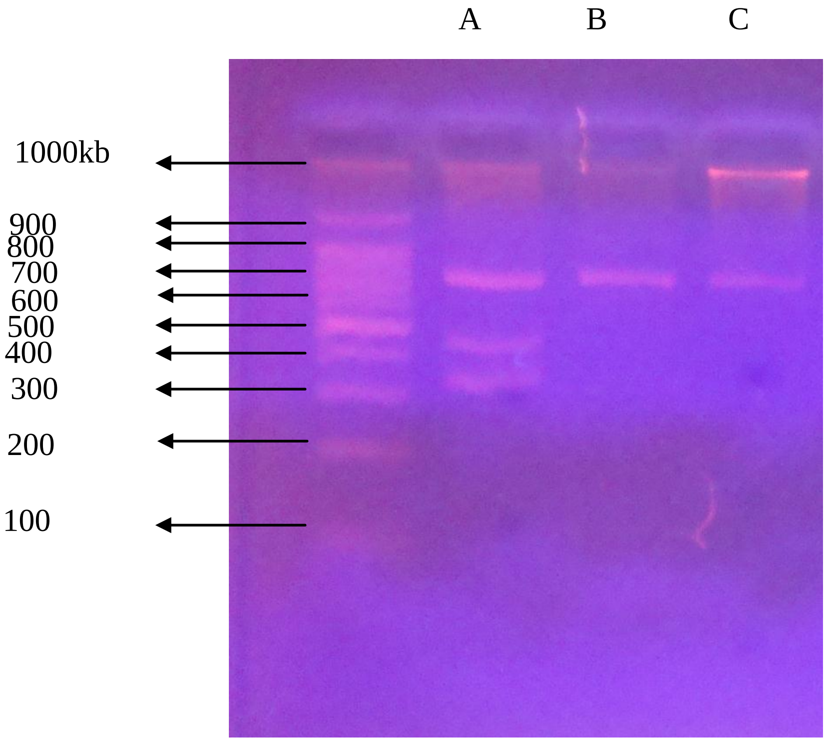

Fig (1): Gel electrophoresis profile of Three isolates of *Rhizobium leguminosarum* (A) isolate carrying four plasmids approximately (1000kb, 700kb, 450kb and 350kb), (B) isolate carrying one plasmid (700kb) and (C) isolate carrying two plasmids (1000kb and 700kb) (Agarose gel:0.8%).

This large number of plasmids distinguishes *R. leguminosarum* from the other rhizobia (1). Soil bacteria from the genus *Rhizobium* are characterized by a complex genomic architecture comprising chromosome and large plasmids. Genes responsible for symbiotic interactions with legumes are usually located on one of the plasmids, named symbiotic plasmid (21). *R. leguminosarum* bv. viciae UPM791, the hydrogenase gene cluster (*hupSLCDEFGHIJK hypABFCDEX*) is located in the large pSym plasmid, which also contains the *nif* and *fix* genes (22). *Rhizobium* field isolates have the unusual feature of harbouring several plasmids, ranging in size from 100 kb to 1000 kb (23). A number of strains of *Rhizobium leguminosarum* biovar *trifolii* was examined for variation in Sym plasmid size and number, using an *R. leguminosarum nifA/nod* probe. Size variation was great in a large proportion of strains, multiple Sym plasmids were observed. A probe containing a repeated sequence (RtRS) incorporating the *nifH* promoter, reported to be specifically found on the Sym plasmid of this biovar, was used to determine whether Sym plasmids were of *trifolii* origin. Strains carrying greater amounts of symbiotic DNA in the form of multiple Sym plasmids(24).

Bacterial genes mediating root attachment, infection, nitrogen fixation, and, with one exception host specificity are clustered in the plasmid-encoded *sym* region. Although non-*sym* plasmids may participate in the symbiosis, the *sym* plasmid alone confers legumespecific (25). Other studies showed that *R. leguminosarum* strain 300 contains at least six large plasmids, one of them pRLlOJI carries genes concerned with nodulation and nitrogen fixation (26,27) .

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مجلة البصرة للعلوم الزراعية 2012، 25(2): 27-33

مقارنة المحتوى البلازميدي لثلاث عزلات لـ *Rhizobium leguminosarum* المعزولة من نبات الباقلاء من مناطق بيئية مختلفة في العراق

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الخلاصة. لوحظ في هذه الدراسة وجود اختلاف في حجم وعدد البلازميدات لثلاث عزلات لجرثومة *Rhizobium leguminosarum* المعزولة من العقد الجذرية لنبات الباقلاء من مناطق مختلفة من العراق .تم مقارنةالمحتوى البلازميدي لهذه الجرثومة حيث دلت النتائج على احتواء احدى العزلات المعزولة من محافظة الناصرية على اربع بلازميدات بوزن جزيئي يتراوح تقريبا من350–1000 كيلوزوج قاعدة واظهرت العزلة الاخرى المعزولة من محافظة السماوه احتواءها على بلازميد واحد بوزن جزيئي تقريبا 700 كيلو زوج قاعدة بينما احتوت العزلة الثالثة المعزولة من محافظة ميسان على بلازميدين بوزن جزيئي تقريبا(700و 1000)كيلوزوج قاعدة هذا يدل على ان هنالك اختلاف في التنوع البلازميدي بين العزلات التي تم الحصول عليها من ترب بيئية مختلفة لذلك ظهر تغاير وراثي بين العزلات المدروسة.