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DNA Sequence Homology in *Rhizobium meliloti* Plasmids

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Summary. Plasmids were recovered by an alkaline procedure from six symbiotically effective strains of *Rhizobium meliloti* of diverse geographical origin, reported to harbour only one middle-size large plasmid (ranging from 89 to 143 Megadaltons). Each purified plasmid was digested with eight restriction endonucleases; cleavage patterns were very complex: only *Kpn*I and *Xba*I gave a limited number of bands. Fingerprints were very different, whatever the restriction enzyme or the geographical origin of the strains. However, Southern DNA-DNA hybridizations revealed that the plasmids showed homologous sequences having a high thermal stability. We gave evidence that some of these sequences are common to all the plasmids of *R. meliloti*. The biological function of these common sequences is unknown. Hybridization with cloned nitrogen fixation (nif) genes from *Klebsiella pneumoniae* had demonstrated that *nif* genes were not located on the middle – size plasmids of *R. meliloti* studied in this paper.

Introduction

The bacterial family of *Rhizobiaceae* comprises two genera, *Agrobacterium* and *Rhizobium*. The bacteria of both genera are able to induce plant cell proliferation. The presence of large plasmids (molecular weight higher than 80 Megadaltons (MD)) is a general feature in the *Rhizobiaceae* family (Zaenen et al. 1974; Casse et al. 1979a, b) and their presence has been correlated with some biological effects of the bacteria on the host plant. These include crown-gall induction by *Agrobacterium tumefaciens* (Van Larebeke et al. 1974; Watson et al. 1975), proliferation of root hairs by *Agrobacterium rhizogenes* (Moore et al. 1979; White and Nester 1980) and establishment of a nitrogen-fixing symbiosis with legumes by *Rhizobium* (Johnston et al. 1978; Casse et al. 1979a; Gross et al. 1979).

Most of the *Rhizobium* species harbour several plasmids, their molecular weights ranging from 50 to 200 MD, some of them being very large: more than 250 MD (Nuti et al. 1977; Casse et al. 1979a). Genetic studies have given evidence that at least some of the genes required for effective symbiosis are plasmid-borne: the host specificity moved together with the transfer of a plasmid from *R. leguminosarum* to *R. trifolii* (Johnston et al. 1978); and the loss of virulence was associated with the loss of a large plasmid in *R. leguminosarum* (Prakash et al. 1979) and *R. meliloti* (Rosenberg, personnal communication). Nevertheless, very little is known about the molecular structure of *Rhizobium* plasmids. Up to now, only their length (Casse et al. 1979a) and some restriction patterns (Ledeboer 1978; Casse et al. 1979b; Prakash et al. 1979; Adachi and Iyer 1980) have been published.

R. meliloti is an endosymbiont of the genera *Trigonella*, *Melilotus* and *Medicago* (alfalfa). In this paper, we analyse the molecular structure of the plasmids of some symbiotically effective strains of *R. meliloti* chosen because of their various geographical origins, previous genetic studies, agricultural importance for alfalfa seed inoculation. From these strains only one plasmid can be isolated as supercoiled DNA molecule by the alkaline procedure used. We compare the isolated plasmids by means of restriction endonuclease digests and Southern hybridizations. We demonstrate the presence of a significant level of sequence homology between the plasmids. Some of the homologous sequences are common to several plasmids. The biological function of these sequences is discussed in relation to symbiotic functions.

Material and Methods

1. Bacterial Strains and Growth Conditions

The *Rhizobium meliloti* strains used are described in Table 1. They were all provided by Dr. Dénarié (INRA Versailles, France). The bacteria were grown on Nutrient Agar: Nutrient Broth 8 g/l, Agar 20 g/l at 31° C. Liquid cultures were grown on TY medium (Beringer 1974): 5 g/l Bactotryptone, 3 g/l Yeast extract, 0.9 g/l $CaCl_2$ pH 7.2 with shaking at 31° C.

2. Restriction Endonucleases

Restriction endonucleases *Eco*RI, *Xba*I, *Xho*I, *Kpn*I were purchased from New England Biolabs and *Hind*III, *Sma*I and *Bam*HI from Boehringer. The reaction conditions used were those recommended by the vendor. *Sal*I was isolated in the laboratory by Dr. Quétier, assay conditions were: 30 mM Tris-HCl pH 7.9, 3 mM $MgCl_2$, 72 mM NaCl, 37° C.

3. Plasmid Isolation Procedures

Bacteria were grown to stationary phase, centrifuged and washed with TE buffer (50 mM Tris-HCl pH 8, 20 mM EDTA). The method used for the isolation of plasmid DNA was derived from Currier and Nester (1976) as modified by Dr. M.D. Chilton (personnal communication).

Bacteria (1.5 g wet weight) were suspended in 85 ml of TE buffer, then lysed by addition of 5 ml of 20% SDS and 10 ml of Pronase 5 mg/ml for 20 min at 37° C. Pronase (Calbiochem grade B) was self-

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Table 1. This table gives the geographical origin and the size of the plasmids of *R. meliloti* studied in this paper

Location of original isolate	Strains	Plasmid molecular weight[a] (MD)
Europe	L5-30	89
	41	143
North America	102F51	100
	12	106
	54032	104
New Zealand	1322	118

[a] Values quoted from gel electrophoresis measurements of Casse et al. (1979a)

digested for 90 min at 37° C. The pH of the lysate was brought up to 12.45 by adding dropwise 3N NaOH while mixing carefully with a spatula. The pH-meter (Metrohm) was previously standardized with Beckman Standard pH 12.45 buffer. Once the desired pH was reached, the lysate was left undisturbed for 10 min at room temperature, then brought to pH 8.5 by adding 20 ml of 2 M Tris pH 7 and left for 15 min at 0° C. NaCl was added to 1 M and the solution was left for 1 h at 0° C. Proteins and aggregated chromosomal DNA were removed by centrifugation (10 min at 15,000 × *g*). A 50% (w/v) solution of polyethylene glycol 6.000 was added to the supernatant up to a final concentration of 10%; plasmid DNA was left to precipitate overnight at 0° C.

Plasmid DNA was collected by low-speed centrifugation (1,000 × *g*, 20 min) and dissolved in TE buffer. The plasmid content of the DNA solution was checked by Tris-Borate agarose gel electrophoresis according to Meyers et al. (1976) and Casse et al. (1979a). DNA was purified by CsCl-ethidium bromide (EtBr) equilibrium density gradient centrifugation (Radloff et al. 1967) carried out for 48 h in a Beckman fixed-angle 50 Ti rotor at 42,000 rpm, 20° C. The DNA bands were visualized under long-wave ultraviolet light (360 nm) and the supercoiled DNA band was removed with a Pasteur pipette. Ethidium bromide was eliminated according to Radloff et al. (1967) and the DNA concentrated by pelleting in a Rotor 50 Ti (45,000 rpm, 16 h at 20° C). Pelleted plasmid DNA was dissolved in TE' buffer (50 mM Tris pH 8, 0.5 mM EDTA) and stored at 4° C. pSA 30 plasmid DNA was prepared by the cleared lysate technique (Clewell and Helinski 1969) by Dr. Boistard (INRA. Versailles. France).

4. Agarose Gel Electrophoresis and Membrane Binding

Electrophoresis was carried out in a vertical slab gel apparatus, with 4 mm thick gels. 0.7 to 1.2% (w/v) agarose (Sigma) was dissolved in TEA electrophoresis buffer (50 mM Tris Acetate pH 8.05, 20 mM Na Acetate, 18 mM NaCl. 2 mM Na_2 EDTA) and samples were run at 2.5 volts/cm for 15 h. After migration, gels were stained with ethidium bromide (2 µg/ml) in TEA for 30 min in the darkness; gels were illuminated with ultraviolet light and photographed with a Polaroïd MP 4 apparatus using a no. 25 Wratten filter (Kodak).

For measuring molecular weight of fragments, λ phage *Hind*III fragments (New England Biolabs). whose molecular weights are well known (Fiandt et al. 1977). were co-migrated on the gels. For high molecular weight fragments (more than 15 MD) RP4 restriction fragments were co-migrated on low concentration agarose gels (0.6%). The *E. coli* strain harbouring RP4 was provided by Dr. Brevet (Inst. Microbiologie Orsay, France); RP4 plasmid was isolated according to the procedure used for *Rhizobium* plasmids.

Multiple bands were resolved by scanning the negative slides with a Joyce-Loeble microdensitometer.

After photographing the bands pattern, the gel was exposed to ultraviolet light for 30 min to reduce the molecular weight of the large fragments and thus increase the efficiency of elution (Deniss and Morgan 1976). The DNA fragments in the gel were transfered to nitrocellulose sheets (Schleicher and Schüll) as described by Southern (1975) and modified by Ketner and Kelly (1976). The efficiency of transfer was checked by rehydrating and staining the gel. Blotted nitrocellulose sheets were dried at room temperature and baked overnight at 60° C under vacuum.

5. In vitro Labelling of DNA

Pure plasmid DNA (1–2 µg) was labelled by the nick translation reaction (Maniatis et al. 1975) employing unlabelled dCTP and dTTP (Boehringer Mannheim) and α [^{32}P]-dATP and α[^{32}P]-dGTP (Radiochemical Centre, Amersham, 3,000 Curies/mmole). DNA polymerase and DNAase were purchased from Boehringer Mannheim. The specific activity of labelled fragments was usually 5–40 × 10^6 cpm/µg with an incorporation efficiency of 40%.

6. Southern Hybridizations

Prior to hybridization, the nitrocellulose membranes were soaked in 0.02 g/l each of Ficoll, bovine serum albumin and Polyvinylpyrrolidone in 6 × SSC, (SSC = 0.15 M NaCl, 0.015 M Trisodium citrate) for 2 h at 37° C (Denhart 1966) and vacuum dried overnight at 60° C.

The membranes were cut lengthwise 0.3 cm wide. Lyophilized radioactive DNA was dissolved in hybridization medium 2 × SSC, 50% formamide, 10 µg/ml Salmon sperm DNA and denatured 10 min at 100° C. Nitrocellulose transfer strips were wet with denatured probe (1 × 10^6 cpm/strip), then overlaid with paraffin oil. Hybridization was carried out at 40° C for 20 h (Roussel and Chabbert 1978).

After hybridization, strips were extensively washed in 2 × SSC at room temperature before drying. The strips were then autoradiographed for 1 to 15 days at −20° C using Rapid R films (Ilford) and Ilford fast tungstate intensifying screens (Laskey and Mills 1977).

Autoradiographic spots were assigned to a restriction band by comparing autoradiographs and photographs of the gel enlarged to the scale 1:1.

In some cases, hybridized nitrocellulose strips were incubated with 25 mM Na acetate buffer pH 4.5, 1 mM $ZnSO_4$, 90 mM NaCl, Nuclease S_1 (Sigma): 10 units, 30 min at 37° C (Bazetoux et al. 1978) and extensively washed with 2 × SSC before autoradiography.

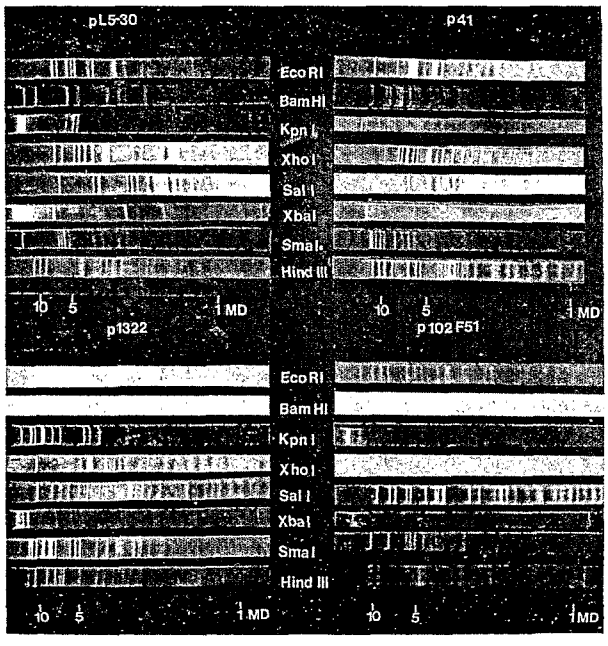

Fig. 1. Cleavage patterns of four different strains of *Rhizobium meliloti*: pRme L5-30, pRme 41, pRme 1322 and pRme 102F51 with the restriction endonucleases indicated. The reaction conditions and method of electrophoretic separation of digests are detailed under Materials and Methods. The mobility of the fragments of the different restriction patterns cannot be compared exactly because the photoprints are derived from different gels and differ in magnification

Results

I. Bacterial Strains

The *Rhizobium meliloti* strains studied (Table 1), effective in nitrogen-fixing symbiosis, are of diverse geographical origins and only one plasmid can be isolated by the alkaline denaturation procedure. The molecular weights of the plasmids range from 89 to 143 Megadaltons (MD) (Casse et al. 1979a).

II. Restriction Patterns

A total of eight restriction endonucleases were screened using plasmid DNA of each of the strains to be analysed.

The Fig. 1 shows the fingerprints of pRme L5–30, pRme 41, pRme 102F51 and pRme 1322 after digestion with *Eco*RI, *Hind*III, *Sma*I, *Kpn*I, *Sal*I, *Xho*I, *Bam*HI and *Xba*I restriction endonucleases. Number, molecular weight and multiplicity of the bands have been measured in each case (data not shown, Jouanin 1980). Table 2 gives an example with *Hind*III fingerprints.

We have not made a systematic search for fragments of molecular weight lower than 1 MD, neithertheless summing up the molecular weights of the bands gives a good approximation of the plasmid length.

As shown in Fig. 1, all restriction patterns are different and complex. *Eco*RI, *Hind*III and *Bam*HI digests give a great number of bands with an upper limit of 15 MD. *Hind*III digests give rise to 50 fragments of more than 0.54 MD with the 143 MD plasmid of strain 41. *Sal*I and *Xho*I give a large number of bands, the molecular weight of which were mainly below 5 MD. *Sma*I which was reported to give a limited number of bands in *Agrobacterium* Ti plasmids (Sciaky et al. 1978), gives only slightly fewer bands than *Eco*RI or *Hind*III. For instance pRme L5–30 (89 MD) is cleaved into 19 bands of molecular weight higher than 1 MD with *Sma*I, but into 24 with *Bam*HI, 20 with *Eco*RI and 25 with *Hind*III.

Plasmids have a small number of *Kpn*I and *Xba*I restriction sites. With *Kpn*I pRme L5–30 (89 MD) is cut into about ten fragments. With *Xba*I, which gives the largest fragments, almost

Table 2. Molecular weights of the pRme L5–30, pRme 41, pRme 1322, pRme 102F51 and pRme 12 *Hind*III digest fragments

Strains	L5–30		41		1322		102F51		12	
Fragments number	M W MD	Multiplicity	M W MD	Multiplicity	M W MD	Multiplicity	M W MD	Multiplicity	M W MD	Multiplicity
1	12.4	1	13.8	1	14.0	1	13.6	1	12	1
2	10.4	1	11.3	1	11.4	1	11	1	10.5	1
3	9.5	1	9.6	2	8.5	1	10.5	1	9	1
4	4.9	1	8.2	1	7.5	1	8.8	1	5.95	1
5	4.7	2	5.9	1	6.25	1	6.15	1	5.75	1
6	4.05	1	5.8	1	6.05	2	4.25	2	5.15	1
7	3.8	1	5.2	1	4.9	1	4.05	1	4.85	1
8	3.55	1	4.85	1	3.9	1	4.0	1	4.2	1
9	3.25	1	3.8	1	3.6	1	3.1	1	4.1	1
10	3.2	1	3.7	1	3.25	1	2.8	2	3.8	1
11	2.95	1	3.5	1	3.05	1	2.7	1	3.6	1
12	2.4	1	3.25	3	2.9	1	2.45	1	3.2	1
13	2.3	2	3.15	1	2.7	1	2.37	1	2.95	1
14	2.05	1	3.10	2	2.55	1	2.12	4	2.75	1
15	1.9	1	3.0	1	2.5	1	1.47	1	2.35	1
16	1.7	1	2.85	1	2.45	1	1.4	1	2.27	1
17	1.4	1	2.75	1	2.2	1	1.27	1	1.85	1
18	1.3	1	2.55	1	2.05	1	1.22	1	1.75	2
19	1.25	1	2.15	2	1.75	1	1.15	1	1.55	1
20	1.2	1	2.1	1	1.7	1	1.05	2	1.50	2
21	1.1	1	2.0	1	1.6	1	0.94	2	1.37	1
22	1.03	1	1.9	1	1.5	2	0.87	1	1.33	1
23	1.0	1	1.8	2	1.42	1	0.8	1	1.29	1
24	0.90	1	1.7	1	1.35	1			1.20	1
25	0.76	1	1.6	1	1.30	1			1.08	2
26	0.66	1	1.4	2	1.27	1			1.04	1
27	0.64	1	1.3	2	1.22	1			0.95	1
28	0.59	2	1.2	1	1.17	1			0.91	1
29			1.12	2	1.15	1			0.78	1
30			1.05	1	1.03	1			0.75	1
31			0.85	1	1.00	1				
32			0.80	1	0.95	1				
33			0.76	1						
34			0.70	2						
35			0.64	1						
36			0.57	1						
37			0.54	1						
Total	91.1		148.09		115.71		103.46		104.06	

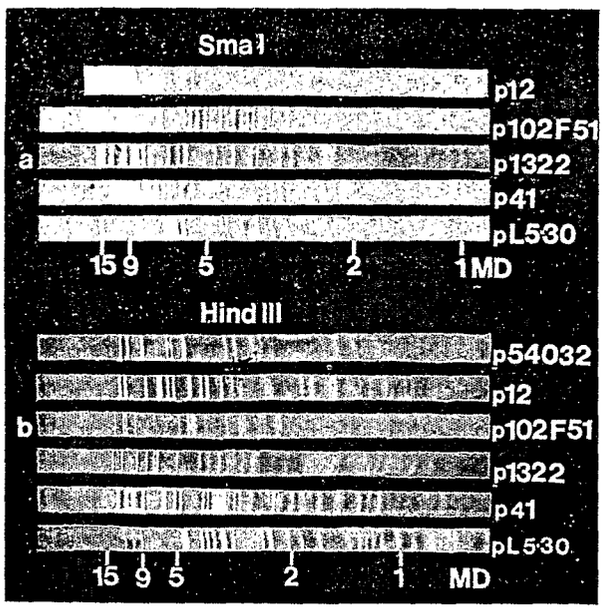

Fig. 2a, b. Comparison of the restriction enzyme digest patterns of plasmids from strains of *Rhizobium meliloti*. **a** *Sma*I digests. **b** *Hind*III digests. The digests were analysed on 1% agarose gels

33% of pRme 41 (143 MD) yields two fragments of 21 and 24 MD; six fragments ranging from 24 to 14 MD represent 72% of pRme 1322 (118 MD) after digestion with *Xba*I.

The comparison of fingerprints of several strains with the same enzyme gives evidence that restriction patterns are markedly dissimilar (Table 2 and Fig. 2). We could never observe any band of the same molecular weight common to more than three of the plasmids listed in Table 1. Furthermore, if we compare strains of close geographical origin (pRme L5–30 and pRme 41 from Europe, for instance) they share very few bands of the same molecular weight but do not have more bands in common than strains of distant geographical origin (pRme L5–30 and pRme 1322, from Europe and New Zealand respectively). This observation has led us to conclude that bands of same molecular weight in the *R. meliloti* plasmid fingerprints are just statistical events and do not reflect sequence conservation.

III. DNA-DNA Hybridization

We looked for DNA sequence homology by DNA-DNA hybridization. Our strategy was to hybridize nick translated labelled plasmid DNA probes with restriction digests of plasmid DNA transferred to cellulose nitrate sheets. From the position and intensity of the spots as revealed by autoradiography, we proposed to identify and estimate the extent of homology between plasmids.

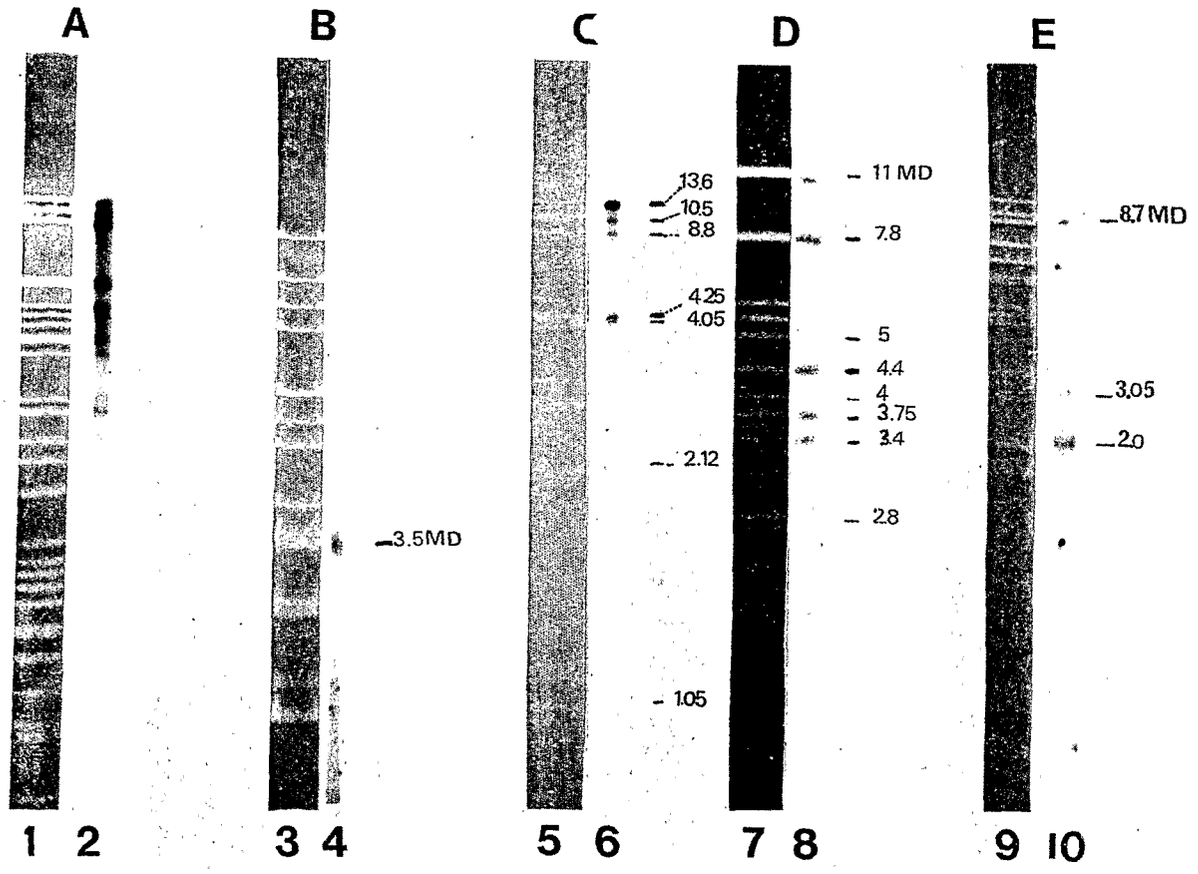

Fig. 3 A–E. Interplasmid hybridization reactions. Restriction endonuclease digests were fractionated on agarose gels, transferred to nitrocellulose sheets and hybridized against ^{32}P-labelled plasmid DNA as probe. **A** 1 pRme L5–30, *Hind*III digest. 2 Autoradiogram after hybridization of pRme L5–30 probe with 1. **B** 3 pRme 102F51, *Eco*RI digest. 4 Autoradiogram after hybridization of pRme 41 probe with 3. **C** 5 pRme 102F51, *Hind*III digest. 6 Autoradiogram after hybridization of pRme 12 probe with 5. **D** 7 pRme 102F51, *Sma*I digest. 8 Autoradiogram after hybridization of pRme L5–30 probe with 7. **E** 9 pRme 1322, *Eco*RI digest. 10 Autoradiogram after hybridization of pRme 41 probe with 9

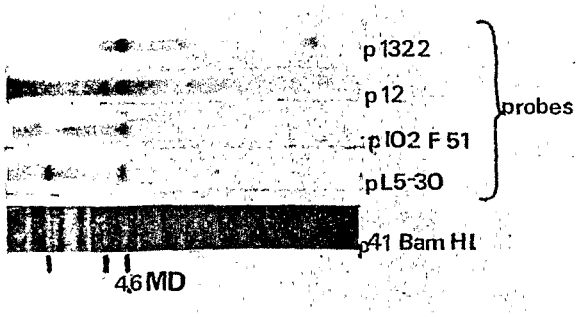

Fig. 4. Hybridization of several probes with pRme 41, *Bam*HI digest as driver. Only the molecular weight of the common sequence have been shown

a) Plasmids Share Homologous Sequences. The experimental conditions of hybridization have been verified by doing homologous hybridizations (Fig. 3A). All the bands were hybridized and the band intensity on the autoradiogram was roughly proportional to the blotted DNA concentration.

Plasmids of the strains 41, L5–30, 102F51, 12, 54032, 1322 were cross-hybridized either as probe or driver. In all experiments, hybridization bands on autoradiographs have been observed, thus giving evidence that sequence homology is general in *R. meliloti* plasmids studied.

Figure 3 shows some typical heterologous hybridizations: pRme 41 probe hybridizes only with band number 10 of the pRme 102F51, *Eco*RI digest and the pRme 12 probe hybridizes with varied intensity to several bands of the pRme 102F51, *Hind*III digest and as does the pRme L5–30 probe with pRme 102F51, *Sma*I digest or pRme 41 probe with pRme 1322, *Eco*RI digest. The quantitative interpretation of these data in terms of the extent of homology is quite difficult because we were not working under conditions of standard DNA reassociation. We did not control the size of the probe, the size of the homologous sequence nor the relative efficiency, so the data can only be analysed on a semi-quantitative basis.

In every experiment, the positions and relative intensities of hybrid bands was unaffected by the solvent: 2 × SSC, 50% formamide 40° C (Roussel and Chabbert 1978), 4 × SSC, 40% formamide, 0.1% SDS, 50° C (Hepburn and Hindley 1979) or 2.5 × SSC, 64° C (Chilton et al. 1978) gave identical results. Using low stringency conditions: 6 × SSC, 60° C or 50° C, the results were unaffected, except for a decrease in the efficiency of hybridization.

The presence of unhybridized single strand tails of the probe may modify the interpretation of the autoradiograms. The obvious method of removing single strand DNA tails is to use nuclease S_1 treatment (Angerer et al. 1975; Bazetoux et al. 1978). Nuclease S_1 treatment reduced the absolute levels of intensity of the major bands but did not modify their relative intensity. Stringent washing conditions such as with 0.01 × SSC at 50° C never affected the autoradiographic results, thereby giving evidence that the hybrids are highly homologous.

We conclude, therefore, that hybridized sequences are due to homologous sequences and as a first approximation that the relative intensity of labelling reflects the length of the hybridized sequences. The pRme 102F51 probe hybridizes to band 2 (12.3 MD) of the pRme 41, *Sma*I digest (data not shown) and to the band 10 (4.6 MD) of the *Bam*HI digest of pRme 41 (Fig. 4). Reciprocally, pRme 41 probe only hybridizes to the band 10 (3.5 MD) of the pRme 102F51, *Eco*RI digest (Table 3).

Table 3. Extent of homology of pRme 102F51, *Eco*RI digested, with other plasmids

Fragment number	M W (MD)	Probes			
		1322	L5–30	41	12
1	17.0	−	−	−	−
2	12.2	+	++	−	−
3	10.0	++	−	−	+
4	8.2	+	++	−	−
5	5.7	−	+	−	++
6	5.1	+	−	−	+
7	4.7	−	−	−	+
8	3.9	−	+	−	−
9	3.6	−	−	−	−
10	3.5	++	++	++	++
11	3.0	−	−	−	−
12	2.95	−	−	−	−
13	2.43	−	−	−	−
14	2.26	+	+	−	−
15	1.77				

− = no hybridizing band
+ = hybridizing band of low intensity
++ = hybridizing band of strong intensity

In this example, homologous sequences between plasmids of strains 41 and 102F51 are clustered on one restriction band but in the majority of hybridizations the labelling is dispersed throughout several restriction bands.

Experiments on thermal denaturation of DNA hybrids on nitrocellulose filters have revealed that the heterologous hybrids did have a high thermal stability suggesting a low sequence divergence between *R. meliloti* plasmids (data not shown). Preliminary data from reassociation experiments indicate that at most 16–20% of the pRme 41 DNA (21–28 MD) is homologous to pRme L5–30, pRme 102F51 and pRme 1322 strains. Unfortunately, we cannot compute the percentage of plasmid length homologous to the hybridized bands, because summing up the hybridized blotted bands would give an overestimate of the percentage of homology. Summing all the hybridized bands always gave values lower than 30 MD whichever driver or probe is used.

b) Occurrence of Conserved Common DNA Sequences in Plasmids. The hybridization of the blotted pRme 102F51, *Eco*RI digest with the probes pRme 12, 41, L5–30, 102F51 or 1322 are compared in Table 3. The pRme 41 probe only hybridizes to band 10 (3.5 MD) of the pRme 102F51, *Eco*RI digest, while pRme L5–30, pRme 1322 and pRme 12 hybridize to several restriction bands but, in any case, band 10 is strongly hybridized (Table 3). Therefore, the five plasmids appear to share DNA homologous to a DNA sequence located in pRme 102F51 DNA at band 10 of the *Eco*RI digest. Figure 4 shows similar experiment using the pRme 41, *Bam*HI digest as blotted DNA and plasmid DNA from 102F51, 12, L5–30, 1322 strains as probes. With each probe, several bands are hybridized with varied intensity but only the band 10 (4.6 MD) hybridizes in each experiment. DNA sequences in band 10 of pRme 41, *Bam*HI digest have been conserved identical in the five strains of *R. meliloti* studied even those of distant geographical origin.

In any experiment whatever the blotted DNA, we could observe one or two restriction bands sharing conserved DNA which hybridize strongly with all the probes tested.

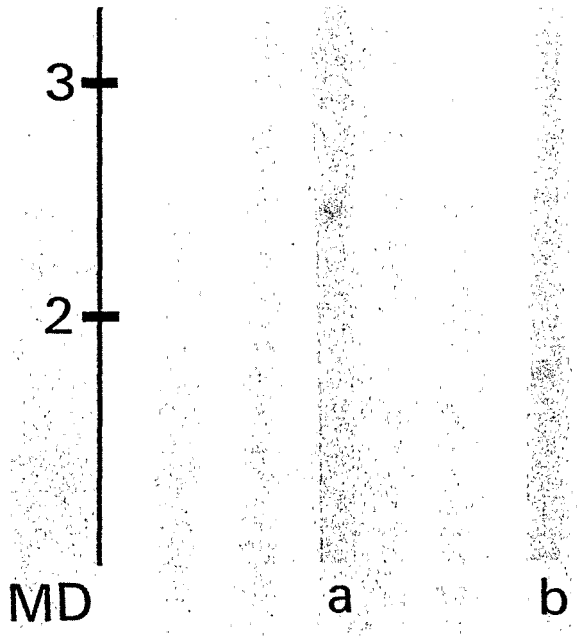

Fig. 5a, b. Autoradiograms after hybridization of the ^{32}P pSA 30 plasmid probe with: a Total DNA Rme 102F51, *Eco*RI digest. b Total DNA Rme 102F51, *Sal*I digest

c) Where are Located nif Structural Genes? Nick translated pSA30 plasmid (Cannon et al. 1979), carrying structural *nif* genes from *Klebsiella pneumoniae*, was hybridized with blotted restriction digests of purified plasmids from *Rhizobium meliloti*. All the experiments with purified plasmids isolated from each of the strains listed on Table 1, were negative.

However, the experiments were positive when total DNA was digested with restriction endonucleases, blotted and hybridized with pSA30 probe. One *Eco*RI fragment (2.4 MD) and one *Sal*I fragment (1.8 MD) from the strain 102F51 of *Rhizobium meliloti* hybridize with pSA30 *nif* probe (Fig. 5).

Discussion

From the restriction patterns presented, we observe that only *Xba*I and, in some strains, *Kpn*I gave a limited number of bands compared with others restriction enzymes such as *Eco*RI or *Bam*HI. This observation is in agreement with those of Depicker et al. (1980) on pTiC58 of *Agrobacterium tumefaciens*. *Xba*I appears to be a very suitable enzyme for molecular cloning of large fragments of plasmid DNA; unfortunately, many standard vectors such as pBR 322 and RP 4 are devoid of *Xba*I restriction sites. The fingerprints from plasmids with different geographical origins obtained with same restriction enzyme are diverse, no bands of identical molecular weight could be observed.

Southern hybridizations have given evidence that sequence homology did occur between all the plasmids tested whatever the geographical origin of the strain. The homology is concentrated in a few restriction fragments but in the absence of a restriction map, we do not know if the hybridized bands are clustered or not.

We have compared the autoradiographic patterns obtained with different probes on blots with the same plasmid driver DNA. The hybridization patterns were different, but in every case, we could observe that one or two restriction bands of the driver hybridized with all the probes. We may interpret these hybridizations as showing some highly conserved sequences. Actually, we do not know the exact size of the common sequences, because driver restriction bands can only be considered as markers. Whatever the strain studied, each plasmid DNA has sequences which exist in every other plasmid examined. What is the biological function of these highly conserved sequences? In the Ti plasmids of genus *Agrobacterium*, DNA-DNA hybridizations have shown that some sequences have been conserved during evolution, sequences which are essential for oncogenicity (Drummond and Chilton 1978; Depicker et al. 1978). In the *Rhizobium* genus one hypothesis is that they are involved in essential functions, for instance nodule formation or host specific recognition. Anyway, the nitrogenase structural genes are not located on the plasmid studied. This is in opposition with the results of Nuti et al. (1979) on *R. meliloti*. On the other hand, our results with total DNA of the strain pRme 102F51 (Fig. 5) are in agreement with those of Ruvkun and Ausubel (1980) on the same strain. Recent results from Casse et al. (personnal communication) demonstrate that *nif* genes are located on another very large plasmid (more than 200 MD) which cannot be isolated as a supercoïled DNA molecule by the alkaline procedure.

In conclusion, the comparison of plasmids of several strains of *R. meliloti* have shown that plasmids share a significant DNA homology. The presence of conserved DNA sequences on each plasmid investigated suggest that they carry a common biological function.

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