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Genetics

Genes for the catabolism and synthesis of an opine-like compound in *Rhizobium meliloti* are closely linked and on the Sym plasmid

(symbiosis/nodule compounds/plant–bacterial interaction)

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ABSTRACT **In alfalfa nodules induced by *Rhizobium meliloti* strain L5-30 the compound L-3-*O*-methyl-*scyllo*-inosamine (3-*O*-MSI) is synthesized. This compound is also catabolized specifically by this strain. Its biological properties are therefore similar to the *Agrobacterium* opines. To answer the question whether opine-like compounds ("Rhizopines") play a role in a plant symbiotic interaction, we isolated the genes for the catabolism of 3-*O*-MSI (*moc* genes) and for the induction of its synthesis in the nodule [*mos* gene(s)]. *moc* and *mos* genes were shown to be closely linked and located on the Sym plasmid of L5-30, suggesting that they have co-evolved and may be important in symbiosis. These genes have been cloned into a broad host-range vector that can be mobilized into other *R. meliloti* strains where they are expressed. The location of the *mos* genes in the bacteria extends the opine concept, initially developed for a plant pathological interaction, to a symbiotic one.**

Plant–bacterial interactions are common in soil. The two best-studied examples are the pathological *Agrobacterium*–crowngall interaction and the symbiotic *Rhizobium*–legume interaction. *Agrobacterium* and *Rhizobium* belong to the same family with *Rhizobium meliloti* regarded as being taxonomically the most similar to *Agrobacterium* (1). The pathological and symbiotic states induced by these two genera also have many features in common, including the ability to redirect plant morphogenesis and the presence of large plasmids involved in interaction with the plant (2, 3). Indeed, transfer of such plasmids from *Rhizobium* to *Agrobacterium* or *vice versa* results in expression of some of the plasmid-encoded symbiotic or pathogenic genes in the recipient host (see ref. 4).

With the plant parasitic *Agrobacterium*–crowngall interaction the bacteria induce galls that act as factories to redirect plant metabolites to produce strain-specific, gall-specific compounds, called opines (5), which can be utilized by the inducing strain. The bacteria carry genes for the catabolism of these compounds and genes for their synthesis on the Ti plasmid. In this system the genes for opine synthesis are transferred and integrated, by way of the transferred DNA, into the plant genome (for a review, see ref. 6). The bacteria thus create an ecological niche, giving them a selective advantage over other bacteria. This phenomenon has been termed genetic colonization (7).

In the *Rhizobium*–legume symbiosis plasmids also play an important role with many of the symbiotic genes (*nod, nif, fix*) being on a large plasmid (Sym plasmid) in a number of *Rhizobium* species, including *R. meliloti, Rhizobium leguminosarum*, and *Rhizobium trifolii* (8–11), but not in others, including *Rhizobium loti* (12).

The development of symbiosis is a complex multistage process culminating in the formation of nodules in which bacteroids (differentiated bacteria) fix molecular nitrogen to ammonia. The plant obtains fixed nitrogen and supplies the bacteroids with photosynthetic products to fuel this process. It is generally accepted that the bacteroids cannot utilize the ammonia but the bacteria benefit from the association by enhancing plant growth, which then supports a vigorous *Rhizobium* population, as well as other bacteria, in the rhizosphere (13, 14).

If the benefit to the *Rhizobium* is in the rhizosphere, then it would seem remarkable if the bacteria do not have a system whereby they can create a selective environment giving them an advantage over other bacteria in the soil, as exists with *Agrobacterium*. In this context it is of interest that the presence of a strain-specific, nodule-specific compound in alfalfa (*Medicago sativa*) has been reported (15, 16). This compound, L-3-*O*-methyl-*scyllo*-inosamine (3-*O*-MSI; Fig. 4*A*; M.E.T., unpublished results) is present in nodules elicited by *R. meliloti* L5-30 and is a specific growth substrate for this strain. Its biological characteristics are therefore similar to the *Agrobacterium* opines.

Here, we report that the genes for the catabolism of 3-*O*-MSI (*moc* genes) by the bacteria and the principal gene(s) for the synthesis of this compound [*mos* gene(s)] in the nodule are closely linked and on the Sym plasmid of *R. meliloti* L5-30.

MATERIALS AND METHODS

Strains and Plasmids. *R. meliloti* strain L5-30 and its spontaneous *nod–nif* deletion mutant L5-22 (11) were obtained from J. Dénarié. AK631 is a compact colony morphology variant of *R. meliloti* 41; ZB375 is a *nod–nif* deletion derivative of AK631 with rifampicin and 5-fluorouracil resistance (17). Strains PM2048, PM2084, and PM2129 are described in the text. *Agrobacterium tumefaciens* C58C1 was obtained from C. Koncz. The *Escherichia coli* strains used were HB101 (18) and AK529, a rifampicin-resistant derivative of HB101 (17). The plasmids were pLAFR1 (19), pACYC184 (20), pRK2013 (21), pPH1JI (22), pJB3JI (8), pKSK5 (23), pSUP5011 (24), and pHM5 (25). Recombinant plasmids constructed in this work are shown in Fig. 1. pPM1056 is an R-prime from L5-30 containing *moc* and *mos* genes. pZB778 is an R-prime from L5-30 containing *nod* genes.

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Abbreviations: 3-*O*-MSI, L-3-*O*-methyl-*scyllo*-inosamine; SI, *scyllo*-inosamine; *mos*, 3-*O*-MSI synthesis induction gene(s); *moc*, 3-*O*-MSI catabolism genes; *nod*, nodulation genes; kb, kilobase(s).

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Media and Growth Conditions. *Rhizobium* strains were grown in TY complete medium or GTS minimal medium (23). *E. coli* strains were grown in LB medium (26). Carbon- and nitrogen-free medium was Bergersen mineral medium (27). Antibiotic concentrations were as described (17).

Purification of 3-*O*-MSI. Acid extracts of nodules were prepared and successively purified by cation-exchange chromatography, biological enrichment with C58C1 to remove nonspecific carbon and nitrogen sources, and a further cation-exchange step, essentially as described (5). The following modifications were made: extraction was in 120 mM HCl and biological enrichment was in Bergersen mineral medium. Nodules (30 g per extraction) were harvested from alfalfa plants that had been inoculated with L5-30 and grown in hydroponic spray culture. *scyllo*-Inosamine (*SI*) was chemically synthesized (M.E.T., unpublished data).

Catabolism Studies. Bacteria (final OD_{550}, 0.5) were incubated in 100 μl of Bergersen mineral medium containing 3-*O*-MSI or *SI* (final concentration, ≈200 μg/ml) as either the sole carbon and nitrogen source or, when the medium was supplemented with 0.2% $(NH_4)_2SO_4$, as the sole carbon source only. Incubation was in small plastic tubes for 3 days with vigorous shaking at 28°C. To check for the disappearance of 3-*O*-MSI, a 5-μl sample was taken and spotted onto 3MM Whatman paper, electrophoresed by high-voltage paper electrophoresis in formic acid/acetic acid buffer, and stained with $AgNO_3$ stain (see ref. 28).

Testing for 3-*O*-MSI Production. Strains to be tested for 3-*O*-MSI synthesis-inducing ability were inoculated onto alfalfa (*M. sativa* cv. Cardinal) seedlings in test tubes (23). Nodules were harvested after 3–4 weeks, twice extracted by crushing in H_2O, and centrifuged and the supernatant was lyophilized and resuspended in H_2O (2.5 mg/μl). Equivalent to 10 mg of nodules was electrophoresed and stained with $AgNO_3$.

Microbiological Techniques. Triparental matings (21) were performed on plates as described (29) with selection on minimal GTS medium supplemented with the appropriate antibiotics. Tn*5* mutagenesis was as described (30). Tn*5* mutants were inserted into the bacterial genome by marker exchange (31) using pPH1JI (22). Verification of the exchange event was by Southern blotting (32) of restriction enzyme digests of total DNA from the mutated strain and probing with a nonmutated fragment homologous to that containing Tn*5*. Mobilization of *Rhizobium* plasmids using Tn*5*-Mob was as described (24) with the addition that after the first mating step colonies were screened for insertion of Tn*5*-Mob into the megaplasmids by hybridizing DNA blots of extracted plasmids (33) with a Tn*5* probe (pHM5; ref. 25). To prepare R-prime plasmids carrying a selected DNA region of L5-30, a cloned fragment of the region was mutagenized with Tn*5*. The Tn*5* insertions were mapped; then mutations at the desired positions were inserted into the L5-30 genome. pJB3JI (8) was mated into the purified homogenotes and R-primes were selected by mating the transconjugants with the *E. coli* strain AK529 essentially as described by Banfalvi *et al.* (17). Formation of R-prime plasmids was shown by agarose gel electrophoresis (33).

DNA Techniques. Plasmid DNA was purified by the alkali method (34) and total DNA was isolated as described (25). Separation of DNA fragments on agarose gels and elution of fragments from gels were as described (34). DNA blotting was according to Southern (32); hybridization and washing conditions were as described (35). Nick-translated hybridization probes and cloning procedures were as described (34). A gene library from L5-30 total DNA and a mini clone bank from pPM1056 were made in pLAFR1 as described (19). Deletion mutants of pLAFR1 clones were prepared by a procedure described by Buikema *et al.* (36). Detection of large plasmids was on agarose gels according to Eckhardt (33).

RESULTS

Cloning of the 3-*O*-MSI Catabolism Genes. Total DNA of *R. meliloti* L5-30 was partially digested with *Eco*RI and cloned into the broad host-range cosmid vector pLAFR1 (19); the clone bank was mated, by triparental mating, into strain L5-22 (a spontaneous *nod–nif* deletion strain of L5-30; ref. 11), which does not catabolize 3-*O*-MSI. Colonies were screened for catabolism of 3-*O*-MSI as a sole carbon source by assaying for the disappearance of 3-*O*-MSI from the incubation medium. This reflects not just uptake of 3-*O*-MSI but also its catabolism, as disappearance of 3-*O*-MSI was associated with growth of bacteria. In this way, six clones capable of catabolizing 3-*O*-MSI were isolated and all contained the same plasmid (pPM1012, Fig. 1), which has a 33-kilobase (kb) insert. pPM1012 was further subcloned by deletion mutagenesis using *Eco*RI. The smallest clone, pPM1031, that could catabolize 3-*O*-MSI has a 15.1-kb insert. This insert contains three *Eco*RI fragments (5.4, 1.0, and 8.7 kb; fragments C, F, and A, respectively, Fig. 1). Removal of either of the terminal fragments A or C from pPM1031 (giving plasmids pPM1028 and pPM1052, Fig. 1) results in plasmids that can no longer confer catabolism of 3-*O*-MSI.

In the unlikely event that the observed growth elicited by pPM1031 was not due to 3-*O*-MSI, but to carbon and nitrogen sources remaining after biological purification of 3-*O*-MSI, we further purified 3-*O*-MSI by high-voltage paper electrophoresis and used this in catabolism tests. Strains with pPM1031 could grow on 3-*O*-MSI purified in this way.

pPM1031 not only conferred catabolism of 3-*O*-MSI to the noncatabolizing L5-30 deletion strain L5-22 but also, when transferred to a noncatabolizing wild-type *R. meliloti* (strain

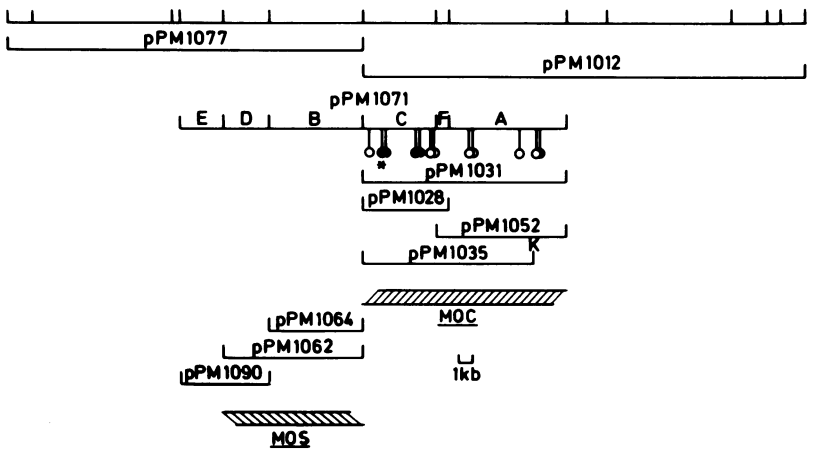

FIG. 1. Physical map of the *moc–mos* region of L5-30. Overlapping cosmids are shown, the top bar being a composite of these. Only *Eco*RI sites are shown (except where indicated, K for *Kpn* I), although the map was confirmed with *Kpn* I digests. All plasmids are in the vector pLAFR1. Deletion mutants were made by partial digestion of the plasmids. The minimum region, as determined by *Eco*RI digestion, for *moc* genes (▨) and *mos* gene(s) (▩) is shown. ♦, Tn*5* insertion giving Moc⁻ phenotype; ♀, Tn*5* insertion giving Moc⁺ phenotype; *, Tn*5* mutant inserted into the L5-30 genome to give PM2129; A–F, *Eco*RI fragments (see text). Tn*5* mutants are shown schematically on pPM1071, although pPM1031 was mutagenized.

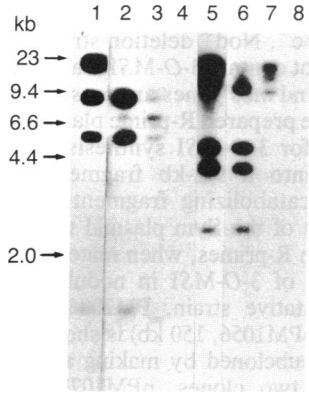

FIG. 2. Hybridization of *Rhizobium* strains with a *moc* probe. Hybridization of the catabolizing plasmid pPM1031 to *Eco*RI digests (lanes 1–4) of plasmid DNA of pPM1012 (lane 1) and total DNA of L5-30 (lane 2), L5-22 (lane 3), and AK631 (lane 4); *Kpn* I digests (lanes 5–8) of plasmid DNA of pPM1012 (lane 5) and total DNA of L5-30 (lane 6), L5-22 (lane 7), and AK631 (lane 8). Autoradiograms of plasmid blots were exposed for 1/10th the time as genomic blots.

AK631), enabled this strain to grow on 3-*O*-M*S*I as a sole carbon source.

The catabolizing plasmid pPM1031 was used to probe total genomic blots of catabolizing and noncatabolizing strains (Fig. 2). Lanes 1 and 5 are controls, in which pPM1031 was probed to plasmid DNA of pPM1012. In lanes 1 and 5 the top band is pLAFR1 and the lower bands are *Eco*RI fragments of 8.7, 5.4, and 1.0 kb (lane 1) and *Kpn* I fragments of 12.4, 5.1, 4.1, and 2.4 kb (lane 5) present in pPM1012. As expected, pPM1031 hybridized strongly to L5-30 total DNA (lanes 2 and 6) but not to the noncatabolizing strains L5-22 (lanes 3 and 7) and AK631 (lanes 4 and 8). The faintly hybridizing bands in the *Eco*RI digest of L5-22 (lane 3) and AK631 (lane 4) that comigrated with the strongly hybridizing bands in L5-30 were not the same fragments as found in L5-30, as shown when the DNA was digested with *Kpn* I (lanes 7 and 8).

The genes for the catabolism of 3-*O*-M*S*I have been designated as *moc* genes.

Localization of the *moc* Genes. Several lines of evidence indicate that the *moc* genes are on the Sym plasmid.

When plasmid gels (33) were probed with a 1.0-kb *moc* probe (fragment F, Fig. 1) the top (megaplasmid) band of L5-30 hybridized (Fig. 3*A*, lane 5). The same band hybridized with an 8.5-kb *nod* probe (pKSK5; ref. 23; Fig. 3*A*, lane 9). No hybridization was observed (Fig. 3*A*, lanes 6 and 7) with the *moc* probe to noncatabolizing strains L5-22 and AK631, indicating the specificity of this probe. The hybridizing band of L5-30 contains two plasmids, seen in Fig. 3*A*, lane 2′, which shows the L5-30 deletion strain L5-22. To see which of these two plasmids (the Sym plasmid or the cryptic megaplasmid; ref. 37) contains the *moc* genes we utilized the Tn*5*-Mob vector system (24) to individually mobilize the plasmids into a *nod*, *nif* deletion strain of AK631 (ZB375; ref. 17) that does not catabolize 3-*O*-M*S*I. A transconjugant strain (PM2048) simultaneously acquired *moc* and *nod* functions, suggesting that the *moc* genes are on the Sym plasmid. This notion was further supported by hybridization of *moc* (Fig. 3*A*, lane 8) and *nod* (Fig. 3*A*, lane 12) probes to the megaplasmid of this strain. Although the incoming Sym plasmid of L5-30 is obscured by a large cryptic plasmid (of a similar size), strain PM2048 is a real transconjugant; its presence is shown as PM2048 hybridizes to the *nod* probe (Fig. 3*A*, lane 12) and nodulates plants (data not shown), whereas ZB375 does neither (17). The deleted *nod–nif* plasmid (second from top, Fig. 3*B*, lane 2) of ZB375 is lost in PM2048 (Fig. 3*B*, lane 3) due to its incompatibility with the incoming Sym plasmid (37), whereas the 140-MDa plasmid remains.

Finally, plasmids of L5-22 (the *nod–nif* deletion strain of L5-30) do not hybridize with *nod* (Fig. 3*A*, lane 10) nor do they hybridize with *moc* (Fig. 3*A*, lane 6), further supporting the Sym plasmid localization of the *moc* genes and suggesting that *nod* and *moc* genes are in the region of the Sym plasmid deleted in L5-22.

Number of *moc* Genes. The insert in pPM1031 required for 3-*O*-M*S*I catabolism is a large fragment (15.1 kb); therefore, we investigated the possibility that this fragment contains several genes. To do this we analyzed the catabolism fragment by deletion and Tn*5* mutagenesis.

Deletion mutants of pPM1031 were prepared by partial digestion with restriction enzymes. These plasmids were then mated into AK631 by triparental mating and tested for the catabolism of 3-*O*-M*S*I. In one such plasmid (pPM1035, Fig. 1), isolated by partial digestion of pPM1012 with *Kpn* I, a 2.4-kb DNA segment from the righthand end of pPM1031 is removed. When mated into AK631, this clone did not catabolize 3-*O*-M*S*I. After electrophoresis of the incubation medium for three times the usual time and staining with $AgNO_3$ two spots could be observed (Fig. 4*B*, lane 3), whereas the control strain containing pPM1031 completely digested 3-*O*-M*S*I (Fig. 4*B*, lane 4). The observed two spots comigrated with 3-*O*-M*S*I and *S*I (Fig. 4*A*), which is a degradation product of 3-*O*-M*S*I. These data suggest that removal of the 2.4-kb fragment from pPM1031 does not inhibit degradation of 3-*O*-M*S*I to *S*I but prevents further catabolism of *S*I. To test this hypothesis we checked whether pPM1035 could catabolize chemically synthesized *S*I as a sole carbon source. Fig. 4*B*, lane 8, shows that pPM1035 could not catabolize *S*I, whereas L5-30 and pPM1031 could (Fig. 4*B*, lanes 7 and 9).

Directed Tn*5* mutagenesis of pPM1031 was performed and the mutated plasmids were transferred to AK631 and tested for 3-*O*-M*S*I catabolism. As shown in Fig. 1, a number of mutants isolated in fragment C, but none of those isolated in

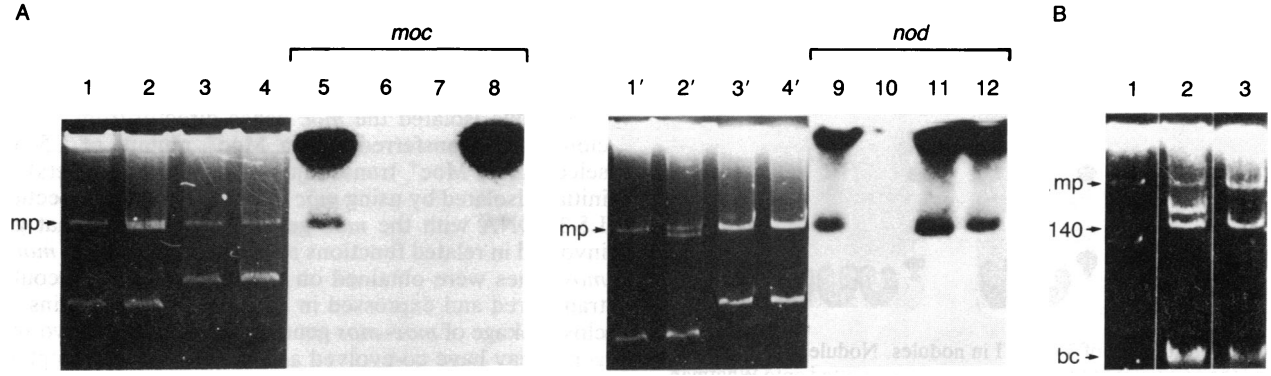

FIG. 3. Localization of *moc* genes. (*A*) Lanes 1–4 and 1′–4′, *R. meliloti* plasmids separated in agarose gel by the procedure of Eckhardt (33); lanes 5–8, Southern blot of the plasmid gel probed with a 1.0-kb *moc* probe (fragment F, Fig. 1); lanes 9–12, Southern blot of the plasmid gel probed with an 8.5-kb *nod* probe (pKSK5; ref. 23). *R. meliloti* strains are L5-30 (lanes 1, 1′, 5, 9), L5-22 (lanes 2, 2′, 6, 10), AK631 (lanes 3, 3′, 7, 11), and PM2048 (lanes 4, 4′, 8, 12). (*B*) *R. meliloti* plasmids separated in agarose gel, L5-30 (lane 1), ZB375 (lane 2), and PM2048 (lane 3). mp, megaplasmid; bc, broken chromosomal DNA; 140, 140 MDa.

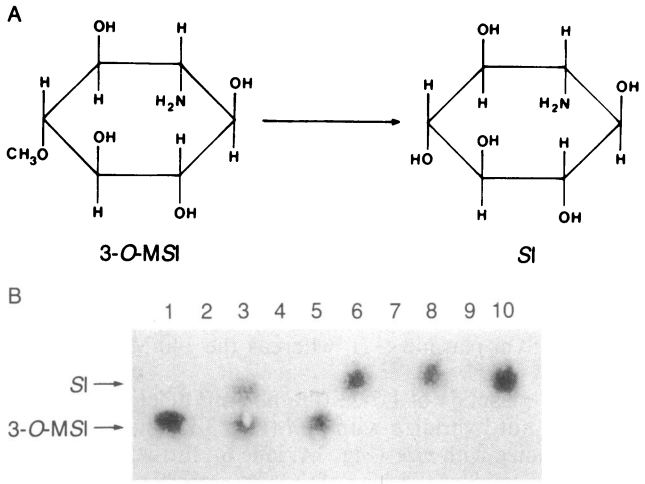

FIG. 4. Structure and catabolism of 3-*O*-MSI and *SI*. (*A*) Structure of 3-*O*-MSI and its degradation product, *SI*. (*B*) Catabolism studies. Lanes 1 and 6, no bacteria (control); lanes 2 and 7, L5-30; lanes 3 and 8, AK631:pPM1035; lanes 4 and 9, AK631:pPM1031; lanes 5 and 10, AK631. Sole carbon source is 3-*O*-MSI (lanes 1–5) and *SI* (lanes 6–10).

fragment A, inhibited 3-*O*-MSI catabolism. The two Tn*5* insertions at the righthand end of fragment A are in the 2.4-kb *Kpn* I–*Eco*RI fragment required for complete catabolism of 3-*O*-MSI. As these insertions do not inhibit catabolism and are 400 base pairs from the *Kpn* I site, this functional region is further mapped to a 2.0-kb region. One of the Tn*5* mutants in fragment C that inhibits 3-*O*-MSI catabolism was inserted into the L5-30 genome by marker exchange (31) and the resulting strain PM2129 could no longer catabolize 3-*O*-MSI.

Isolation and Localization of 3-*O*-MSI Synthesis-Inducing Gene(s). We tested the transconjugant PM2048 for the ability to induce the production of 3-*O*-MSI in nodules. Fig. 5*A*, lane 3, shows that PM2048 induced the production of 3-*O*-MSI in the nodules, whereas a nodulating form of the recipient (AK631) does not (Fig. 5*B*, lane 3). Therefore, 3-*O*-MSI synthesis-inducing gene(s) [designated as *mos* gene(s)] are on the Sym plasmid. We do not know why the level of 3-*O*-MSI is reduced in strain PM2048 compared with L5-30.

Further isolation of *mos* gene(s) was based on the assumption that they are linked to the *moc* genes. This was a reasonable assumption, as when a *nod* R-prime from AK631 was introduced into L5-22 (a Moc$^-$, Nod$^-$ deletion strain of L5-30) the nodules induced did not contain 3-*O*-MSI (data not shown). As this suggested *moc* and *mos* genes are present in the region deleted from L5-22, we prepared R-prime plasmids in this region and tested them for 3-*O*-MSI synthesis. Accordingly, Tn*5* was inserted into a 9.2-kb fragment of pPM1012 to the right of the catabolizing fragments and R-primes carrying large sections of the Sym plasmid tagged with Tn*5* were made. Most of the R-primes, when mated into AK631, induced the production of 3-*O*-MSI in nodules. A nodule extract from a representative strain, PM2084, that contains one of these R-primes (pPM1056, 150 kb) is shown in Fig. 5*B*, lane 4. pPM1056 was subcloned by making a mini cosmid bank in pLAFR1 and two clones, pPM1071 and pPM1077 (Fig. 1), that induced the synthesis of 3-*O*-MSI were isolated. These two plasmids have three *Eco*RI fragments in common (fragments E, D, and B, Fig. 1) and these are to the left but closely linked to the catabolism genes. One of these plasmids (pPM1071) has functioning *moc* and *mos* genes. To determine whether all three *Eco*RI fragments are required for synthesis induction we subcloned pPM1071 by partial *Eco*RI digestion, giving pPM1062, pPM1064, and pPM1090 (Fig. 1), and mated these into AK631 to test for the production of 3-*O*-MSI in the nodules. Only pPM1062 induced the production of 3-*O*-MSI, indicating that *Eco*RI fragments B and D contain the *mos* gene(s).

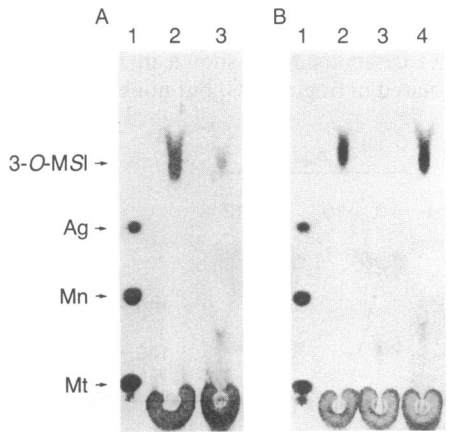

FIG. 5. Detection of 3-*O*-MSI in nodules. Nodules were extracted, and the equivalent of 10 mg of nodules was spotted onto Whatman 3MM paper and, after electrophoresis, was stained with $AgNO_3$. (*A* and *B*) Two different experiments. (*A*) Lane 1, markers: Ag, agropine; Mn, mannopine; Mt, mannitol. Lane 2, L5-30; lane 3, PM2048. (*B*) Lane 1, markers as above; lane 2, L5-30; lane 3, AK631; lane 4, PM2084.

Position of *moc* and *mos* Genes on the Sym Plasmid. An R-prime, pZB778 of 300 kb, made by marking L5-30 host-specificity genes with Tn*5* and which complemented nodulation functions (Z.B., unpublished results), did not catabolize 3-*O*-MSI nor did the R-prime pPM1056 (containing *moc–mos* genes) hybridize with pKSK5, the 8.5-kb *nod* probe (data not shown), suggesting *nod* and *moc–mos* genes are not closely linked. Furthermore, by using overlapping cosmids, prepared from the R-prime pPM1056 and which extended ≈25 kb to the left and ≈40 kb to the right of the *moc–mos* region as probes to pZB778 and pPM1056, we could not find common hybridizing bands. However, L5-22 does not contain *nod* genes or the *moc–mos* genes, implying that all of these genes are on the deleted region of the Sym plasmid in this strain (Fig. 3*A*, lane 2′).

DISCUSSION

Production and selective catabolism of opines plays an important role in the interaction between soil bacteria, such as crowngall-inducing *Agrobacterium*, and its plant hosts. Evidence has been obtained (15, 16) that *R. meliloti* strain L5-30 induces the production of an opine-like product in nodules. As a contribution to answering the question whether or not opines play a more general role in bacterial–plant interactions, the genes involved in the synthesis [*mos* gene(s)] and catabolism (*moc* genes) of this opine-like product, 3-*O*-MSI, were mapped and DNA fragments carrying these genes were isolated from L5-30.

We have isolated the *moc* genes directly from an L5-30 clone bank transferred into a Moc$^-$ mutant of L5-30 by selecting for Moc$^+$ transconjugants. The *mos* gene(s) were initially isolated by using *moc* R-primes carrying a section of L5-30 DNA with the *moc* genes and assuming that genes involved in related functions are clustered. Finally, *moc* and *mos* genes were obtained on a single cosmid and could be transferred and expressed in other *R. meliloti* strains. This close linkage of *moc–mos* genes suggests that the two sets of genes may have co-evolved as a functional unit, implying a degree of importance to the phenomenon.

The *moc–mos* genes have been shown by plasmid mobilization and hybridization studies to be on the *nod–nif* Sym plasmid of L5-30. Although present on the Sym plasmid, the *moc–mos* genes are not located close to the *nod* genes.

However, all of these genes are missing from L5-22, a mutant of L5-30 that has a deletion in the Sym plasmid. The size of this deletion is estimated to be considerably less than half of the Sym plasmid, as it does not dramatically alter the mobility of the plasmid. Nevertheless, the presence of *moc–mos* genes on the symbiotic megaplasmid is in line with our suggestion that these genes are involved in symbiotic function.

By deletion mutagenesis of the original clone we obtained a 15.1-kb fragment that contains the *moc* genes. We have phenotypic evidence for at least two functional catabolic regions on this fragment. One region located in fragment C (Fig. 1), found by Tn*5* insertion, may be involved in the demethylation of 3-*O*-M*S*I to *S*I. The other region at the righthand end of fragment A, the removal of which results in the accumulation of *S*I, may be involved in the further catabolism of *S*I. A number of Tn*5* inserts between these two regions have no effect on 3-*O*-M*S*I catabolism, suggesting that not all of the 15.1-kb fragment is required for catabolism. These data do not exclude the possibility that within the 15.1-kb insert of pPM1031 there are more than two genes.

The presence of 3-*O*-M*S*I is not as universal as the opines of agrobacteria. Aside from L5-30, of 20 other strains tested, only 2 can catabolize it and induce the production of either 3-*O*-M*S*I or the very closely related compound, *S*I, in nodules. It is interesting that one of these is an *R. leguminosarum* strain (P.J.M., unpublished data). In addition, inositol compounds similar to 3-*O*-M*S*I have also been found in nodules induced by *R. leguminosarum* (38). Furthermore, the presence of an unrelated nodule-specific, opine-like compound has been reported in nodules induced by *R. loti* (39). We believe more opine-like compounds ("Rhizopines") may be found in nodules induced by other *Rhizobium* strains and species if a thorough screening were undertaken.

Localization of the *mos* gene(s) in *Rhizobium* extends the *Agrobacterium* opine concept first developed for a pathological interaction to a symbiotic one. In the *Agrobacterium* pathological interaction, the bacteria redirect plant metabolites to produce opines that are specifically catabolized by the inducing bacteria, thus ensuring a selective advantage. We envisage that the presence of opine-like compounds in *Rhizobium* may also be a subtle way for the bacteria to sequester plant or symbiotic metabolites to enhance its benefit in the symbiotic relationship.

Isolation of the genes for catabolism and the primary gene(s) for synthesis of 3-*O*-M*S*I has given us a tool with which we can analyze the mechanism by which the bacterial genes are involved in the synthesis of this compound in the nodule and a tool to analyze the function of this compound in *Rhizobium*.

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1. De Ley, J., Bernaerts, M., Rassel, A. & Guilmont, A. (1966) *J. Gen. Microbiol.* **43,** 7–17.
2. Verma, D. P. S. & Long, S. R. (1983) *Int. Rev. Cytol. Suppl.* **14,** 211–245.
3. Vance, C. P. (1983) *Annu. Rev. Microbiol.* **37,** 399–424.
4. Hooykaas, P. J. J. & Schilperoort, R. A. (1984) *Adv. Genet.* **22,** 209–283.
5. Guyon, P., Chilton, M.-D., Petit, A. & Tempé, J. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2693–2697.
6. Gheysen, G., Dhaese, P., Van Montagu, M. & Schell, J. (1985) in *Genetic Flux in Plants*, eds. Hohn, B. & Dennis, E. S. (Springer, Vienna) pp. 11–47.
7. Schell, J., Van Montagu, M., De Beuckeleer, M., De Block, M., Depicker, A., De Wilde, M., Engler, G., Genetello, C., Hernalsteens, J. P., Holsters, M., Seurinck, J., Silva, B., Van Vliet, F. & Villarroel, R. (1979) *Proc. R. Soc. London Ser. B* **204,** 251–266.
8. Brewin, N. J., Beringer, J. E. & Johnston, A. W. B. (1980) *J. Gen. Microbiol.* **120,** 413–420.
9. Banfalvi, Z., Sakanyan, V., Koncz, C., Kiss, A., Dusha, I. & Kondorosi, A. (1981) *Mol. Gen. Genet.* **184,** 318–325.
10. Hooykaas, P. J. J., Van Brussel, A. A. N., Den Dulk-Ras, H., Van Slogteren, G. M. S. & Schilperoort, R. A. (1981) *Nature (London)* **291,** 351–353.
11. Rosenberg, C., Boistard, P., Dénarié, J. & Casse-Delbart, F. (1981) *Mol. Gen. Genet.* **184,** 326–333.
12. Chua, K.-Y., Pankhurst, C. E., Macdonald, P. E., Hopcroft, D. H., Jarvis, B. D. W. & Scott, D. B. (1985) *J. Bacteriol.* **162,** 335–343.
13. Beringer, J. E., Brewin, N., Johnston, A. W. B., Schulman, H. M. & Hopwood, D. A. (1979) *Proc. R. Soc. London Ser. B* **204,** 219–233.
14. Dixon, R. O. D. (1969) *Annu. Rev. Microbiol.* **23,** 137–158.
15. Tempé, J., Petit, A. & Bannerot, H. (1982) *C. R. Acad. Sci. Paris (Ser. 3)* **295,** 413–416.
16. Tempé, J. & Petit, A. (1983) in *Molecular Genetics of the Bacteria Plant Interaction*, ed. Pühler, A. (Springer, Berlin) pp. 14–32.
17. Banfalvi, Z., Randhawa, G. S., Kondorosi, E., Kiss, A. & Kondorosi, A. (1983) *Mol. Gen. Genet.* **189,** 129–135.
18. Boyer, H. W. & Roulland-Dussoix, D. (1969) *J. Mol. Biol.* **41,** 459–472.
19. Friedman, A. M., Long, S. R., Brown, S. E., Buikema, W. J. & Ausubel, F. M. (1982) *Gene* **18,** 289–296.
20. Chang, A. C. Y. & Cohen, S. N. (1978) *J. Bacteriol.* **134,** 1141–1156.
21. Ditta, G., Stanfield, S., Corbin, D. & Helinski, D. R. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 7347–7351.
22. Beringer, J. E., Beynon, J. L., Buchanan-Wollaston, A. V. & Johnston, A. W. B. (1978) *Nature (London)* **276,** 633–634.
23. Kondorosi, E., Banfalvi, Z. & Kondorosi, A. (1984) *Mol. Gen. Genet.* **193,** 445–452.
24. Simon, R. (1984) *Mol. Gen. Genet.* **196,** 413–420.
25. Meade, H. M., Long, S. R., Ruvkun, G. B., Brown, S. E. & Ausubel, F. M. (1982) *J. Bacteriol.* **149,** 114–122.
26. Miller, H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
27. Bergersen, F. J. (1961) *Aust. J. Biol. Sci.* **14,** 349–360.
28. Dahl, G. A., Guyon, P., Petit, A. & Tempé, J. (1983) *Plant. Sci. Lett.* **32,** 193–203.
29. Kondorosi, A., Kiss, G. B., Forrai, T., Vincze, E. & Banfalvi, Z. (1977) *Nature (London)* **268,** 525–527.
30. De Bruijn, F. J. & Lupski, J. R. (1984) *Gene* **27,** 131–149.
31. Ruvkun, G. B. & Ausubel, F. M. (1981) *Nature (London)* **289,** 85–88.
32. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
33. Eckhardt, T. (1978) *Plasmid* **1,** 584–588.
34. Maniatis, T., Fritsch, E. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
35. Kondorosi, A., Kondorosi, E., Pankhurst, C. E., Broughton, W. J. & Banfalvi, Z. (1982) *Mol. Gen. Genet.* **188,** 433–439.
36. Buikema, W. J., Long, S. R., Brown, S. E., Van Den Bos, R. C., Earl, C. D. & Ausubel, F. M. (1983) *J. Mol. Appl. Genet.* **2,** 249–260.
37. Banfalvi, Z., Kondorosi, E. & Kondorosi, A. (1985) *Plasmid* **13,** 129–138.
38. Skøt, L. & Egsgaard, H. (1984) *Planta* **161,** 32–36.
39. Shaw, G. J., Wilson, R. D., Lane, G. A., Kennedy, L. D., Scott, D. B. & Gainsford, G. J. (1986) *J. Chem. Soc. Chem. Commun.* **2,** 180–181.