Symbiotic characterization of isoleucine + valine and leucine auxotrophs of
*Sinorhizobium meliloti*

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Ten isoleucine+valine and three leucine auxotrophs of *Sinorhizobium meliloti* Rmd201 were obtained by random mutagenesis with transposon Tn5 followed by screening of Tn5 derivatives on minimal medium supplemented with modified Holliday pools. Based on intermediate feeding, intermediate accumulation and cross-feeding studies, isoleucine+valine and leucine auxotrophs were designated as ilvB/ilvG, ilvC and ilvD, and leuC/leuD and leuB mutants, respectively. Symbiotic properties of all ilvD mutants with alfalfa plants were similar to those of the parental strain. The ilvB/ilvG and ilvC mutants were Nod +. Inoculation of alfalfa plants with ilvB/ilvG mutant did not result in root hair curling and infection formation. The ilvC mutants were capable of curling root hairs but did not induce infection thread formation. All leucine auxotrophs were Nod + Fix -. Supplementation of leucine to the plant nutrient medium did not restore symbiotic effectiveness to the auxotrophs. Histological studies revealed that the nodules induced by the leucine auxotrophs did not develop fully like those induced by the parental strain. The nodules induced by leuB mutants were structurally more advanced than the leuC/leuD mutant induced nodules. These results indicate that ilvB/ilvG, ilvC and one or two leu genes of *S. meliloti* may have a role in symbiosis. The position of ilv genes on the chromosomal map of *S. meliloti* was found to be near ade-15 marker.

The bacteria belonging to the genera *Rhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Mesorhizobium* and *Sinorhizobium*, collectively called rhizobia, enter into a symbiotic association with leguminous plants. The symbiotic association is a complex process involving recognition and infection of the host plant, development of root nodules, multiplication of bacteria and their conversion into bacteroids within the plant cells, and finally reduction of molecular nitrogen to ammonia by bacteroids. Several symbiotic genes of rhizobia and legumes have been identified. Certain rhizobial genes of the primary biosynthetic pathways appear to play a role in symbiosis.

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An isoleucine and valine auxotroph of *S. meliloti* strain L5-30 was non-infective. Two isoleucine and valine auxotrophs of *S. fredii* HH303 were found to induce ineffective nodules on soybean. A Tn5-induced isoleucine and valine auxotroph of *S. meliloti* 1021 did not induce nodules on alfalfa. The Tn5 insertion in this mutant was located in the ilvC gene which codes for the enzyme isomeroreductase. The ilvC mutants obtained from different *S. meliloti* wild-type strains have been reported to induce root hair deformation on alfalfa roots. These mutants showed variable activation of the common nodulation genes nodABC; however, all these mutants were Nod +. The leucine auxotrophs of *S. meliloti* have been reported to induce ineffective nodules on alfalfa plants whereas leucine auxotrophs of *B. japonicum* were found to form effective nodules on soybean plants. Truchet et al. carried out histological studies on the nodules induced by leucine auxotrophs. However, the positions of biochemical blocks in these mutants were not known.

It is clear from the above reports that further research work is required to identify the gene(s)/enzyme(s)/intermediate(s) of the isoleucine, valine and leucine biosynthetic pathways of rhizobia.
having a role in symbiosis. We report here the isolation and symbiotic characterization of 10 isoleucine-valine and 3 leucine auxotrophs of *S. meliloti* Rmd201.

**Materials and Methods**

**Bacterial strains, plasmids and plant cultivar**—The bacterial strains and plasmids used in this study are listed in Table 1. The alfalfa (*Medicago sativa* cv. T9) seeds were procured from National Seeds Corporation Limited, Pusa Complex, New Delhi, India.

**Media and growth conditions**—The media used and growth conditions have been mentioned previously.

Tn5 mutagenesis and screening for auxotrophs—Tn5 mutagenesis and screening for isoleucine+valine and leucine auxotrophs were done as described earlier.

Location of biochemical block in each auxotroph—The position of biochemical block in each auxotroph was determined by intermediate feeding, intermediate accumulation and cross feeding methods as described previously. For intermediate feeding studies, a cell suspension of each isoleucine+valine auxotroph was streaked on Rhizobium minimal medium (RMM) supplemented with α-ketobutyrate and valine, α-ace-tolactate and isoleucine, and α-keto-β-methylvalerate and valine; the cell suspension of each leucine auxotroph was streaked on RMM supplemented with α-ketoisovalerate, α-isopropylmalate and α-ketoisocaproate.

Pyruvic acid accumulation in the cultures of all isoleucine and valine auxotrophs was estimated. A sample (10 ml) of log phase culture of each auxotroph was centrifuged at 5000 rpm for 10 min. The pellet obtained was washed twice with liquid RMM (2 ml each time), resuspended in 10 ml of RMM and incubated at 28°C for 48 hrs on an orbital shaker (speed 120 rpm). The resulting bacterial culture was centrifuged at 10,000 rpm for 10 min and the supernatant was analyzed chromatographically for pyruvic acid by the method of Umbarger and Mueller as described in Smith and Pattee. A sample (0.5 ml) of the supernatant was spotted on a chromatographic paper. Pyruvic acid solution (100mg/ml) was used as a standard. The chromatogram was developed (ascending method) for 6.5 hrs in a solvent that consisted of s-butanol-propionic acid (95:5, v/v) saturated with water. Pyruvic acid was detected by spraying the chromatogram with α-phenylenediamine reagent followed by heating at 100°C for 2 min.

Table 1—Bacterial strains and plasmids used/constructed

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<th>Strains/Plasmids</th>
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<tr>
<td>Rmd201</td>
<td>Spontaneous Sm^r^ derivative of AK631 (Nod^+^ Fix^+^)</td>
<td>Khamuja &amp; Kumar</td>
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<td>PP631</td>
<td>AK631(pJB3J1)</td>
<td>Peter Putnoky</td>
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<td>Rmd201 ilvB/IlvC::Tn5</td>
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<td>Brewin et al</td>
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The cross feeding assays were done by streaking different combinations of isoleucine+valine auxotrophs close to each other on RMM agar medium supplemented with isoleucine and valine (2 µg/ml each). Similarly leucine auxotrophs were streaked on RMM supplemented with leucine (2 µg/ml).

**Linkage of Tn5 insertion to auxotrophy**—This was determined for each auxotroph as described earlier.

**Genetic mapping of Tn5 insertions**—The location of transposon Tn5 insertion in each isoleucine and valine auxotroph was determined by plasmid pJB31-mediated mapping method. The donor strain (containing plasmid pJB31) of each auxotroph was mated separately with four S. meliloti mapping strains, viz., ZB555, ZB556, ZB557 and ZB205. Two hundred kanamycin resistant transconjugants from each cross were selected on TY agar medium containing rifampicin (40 µg/ml) and kanamycin sulphate (400 µg/ml). The co-transfer of kanamycin resistance with each unselected recipient marker was determined by streaking these transconjugants on appropriate selective media. The co-inheritance frequency or linkage of the unselected marker for each auxotroph was calculated and the distance between these markers was determined using the formula: c = (1-d)^3, where c is the linkage of two markers, and d is the distance between them.

**Plant inoculation tests**—Alfalfa plants were inoculated with the rhizobial strains as described earlier. Five revertants of each auxotroph were inoculated on alfalfa plants to establish that the defect in symbiosis was due to auxotrophy. Symbiotic interactions of isoleucine and valine auxotrophs with alfalfa plants were also studied using the nitrogen free plant nutrient medium supplemented with isoleucine and valine, and α-keto-β-methylvalerate and valine.

**Light microscopy for observing infection thread formation**—After 4 days of inoculation, the root portion of each plant was washed with distilled water and cut into 1 cm long pieces. These root pieces were placed in methylene blue solution (0.01% w/v in distilled water) for 15 min and washed twice with sterile water. Each stained root piece was placed on a glass slide, covered with a cover slip and examined at 10x magnification under light microscope (Leica DM LB).

**Histology of nodules**—Histology of nodules induced by leucine auxotrophs was studied by the method described previously.

**Results**

**Tn5 mutagenesis and isolation of isoleucine+valine and leucine auxotrophs**—By random mutagenesis of S. meliloti Rm201 with transposon Tn5, 31,673 Tn5-induced kanamycin resistant transconjugants were obtained. Out of these transconjugants 10 were isoleucine+valine and three leucine auxotrophs (Table 1). Each isoleucine+valine auxotroph was able to grow when both isoleucine and valine were added to RMM. The isoleucine+valine and leucine auxotrophs were referred to as ilv (i = isoleucine; v = valine) and leu mutants, respectively. Spontaneous reversion to prototrophy occurred in all these mutants. The reversion frequencies for ilv mutants ranged from 1.2 × 10^{-9} (RHI) to 2.8 × 10^{-9} (RH3) (data for all mutants not presented). The reversion frequencies were 1.4 × 10^{-9}, 1.5 × 10^{-9} and 1.9 × 10^{-9} for the leu mutants RS3, RH14 and SY1, respectively.

**Location of biochemical blocks**—On the basis of intermediate feeding, pyruvic acid accumulation and cross-feeding studies, all ilv mutants were classified into following three categories: (i) ilvB/H/G mutant (VK4) which grew on RMM supplemented with α-acetolactate and isoleucine, accumulated pyruvic acid, and was cross-fed by all other isoleucine and valine auxotrophs, (ii) ilvC mutants (RH1, RH3, SY2 and PS7) which did not grow on RMM supplemented with α-acetolactate and isoleucine, and were cross-fed by RH3, RH18 and VK44 auxotrophs, and (iii) ilvD mutants (RH3, RH18 and VK44) which grew on RMM supplemented with α-keto-β-methylvalerate and valine, and were not cross-fed by any other isoleucine and valine auxotroph. The leucine auxotrophs were placed in the following two categories on the basis of intermediate feeding and cross-feeding studies: (i) leuC/leuD mutant (RS3) which did not grow on RMM supplemented with α-isopropylmalate and was cross fed by RH14 and SY1 auxotrophs, and (ii) leuB mutants (RH14 and SY1) which grew on RMM supplemented with α-ketocisopropionate and cross fed RS3 auxotroph. The position of biochemical block in each leucine auxotroph has been shown in Fig. 1.

**Linkage of Tn5 insertion to auxotrophy and symbiotic defect**—When transposon Tn5-encoded kanamycin resistance marker of each isoleucine+valine and leucine auxotroph was transferred to S. meliloti ZB555 strain, all kanamycin resistant transconjugants (100 in each case) were found to be isoleucine+valine and leucine auxotrophs, respectively. One hundred
per cent co-transfer of Tn5 and auxotrophy indicated complete linkage of transposon Tn5 insertion to auxotrophy. In other words, each auxotrophic cell had only one Tn5 insertion. The revertants of auxotrophs showed, like the parental strain Rmd201, normal symbiosis with alfalfa plants. These results revealed that a single Tn5 insertion in each of these auxotrophs resulted in auxotrophy and symbiotic defect.

Genetic mapping of Tn5 insertions—Transposon Tn5-encoded kanamycin resistance marker of each ilv mutant showed linkage with ade-15 marker. The co-transfer values of kanamycin resistance with ade-15 were 24, 9 and 5% for RH18 (ilvD mutant), RH1 (ilvC mutant) and VK4 (ilvB/ilvG mutant) auxotrophs, respectively, and the map distances between kanamycin resistance and the ade-15 markers were 0.38, 0.55 and 0.63 for these strains, respectively (mapping data of other strains not presented).

Symbiotic properties of auxotrophs—All ilvD mutants (RH3, RH18 and VK44) induced nodules, which were cylindrical in shape and pink in colour, on alfalfa plants. The mean dry weights of the plants inoculated with these mutants did not differ significantly from that of the plants inoculated with the parental strain Rmd201 (data not presented) indicating that the nitrogen fixing efficiencies of ilvD mutants were similar to that of the parental strain. These strains were hence Nod+ Fix+. The ilvB/ilvG mutant
(VK4) and all ilvC mutants (RH1, RH3, VK5, NV29, SY2 and PS7) did not induce nodules. These strains were, therefore, Nod'. The supplementation of the nitrogen free plant nutrient medium with isoleucine and valine, or with α-keto-β-methylvalerate and valine did not restore symbiotic effectiveness to ilvB/ilvG and ilvC mutants. All leucine auxotrophs induced nodules which were round and white. The mean dry weights of the plants inoculated with these auxotrophs did not differ significantly from that of the uninoculated plants (data not presented) indicating that these auxotrophs did not fix nitrogen. The leucine auxotrophs were hence Nod' Fix'. Supplementation of the nitrogen free plant nutrient medium with leucine or α-ketoisocaproate did not restore symbiotic effectiveness to the leucine auxotrophs. Normal symbiosis, like that of the parental strain, was observed when alfalfa plants were inoculated with the spontaneous revertants of leucine auxotrophs.

Some alfalfa plants inoculated with ilv and leu mutants had pink nodules and morphological features like the plants inoculated with the parental strain Rmd201. The nodules on these plants were found to be occupied by the prototrophic revertants. The percentages of plants showing 100% occupancy of nodules by the leucine auxotrophs were 67.5, 72.5 and 60.0 for the mutants RS3, RH14 and SY1, respectively.

**Root hair curling and infection thread formation**

The ilvB/ilvG mutant (VK4) did not induce root hair curling and infection thread formation. The ilvC mutants (RH1, RH30, VK5, NV29, SY2 and PS7) were capable of curling root hairs but did not produce infection threads. The ilvD mutants (RH3, RH18 and VK44) resulted in root hair curling and infection thread formation like the parental strain Rmd201 (Fig. 2).

**Light microscopy of nodules**—A longitudinal cross section of a nodule induced by the parental strain showed a central tissue surrounded by several peripheral tissues which included vascular bundles (Fig. 2A). The central tissue was differentiated into five zones, viz., apical meristematic zone, infection zone, interzone between infection and nitrogen fixation zones, nitrogen fixation zone and senescence zone. The apical meristematic zone contained uninfected and constantly dividing nodule cells. Infection threads were seen in intercellular spaces of the nodule cells in the infection zone (Fig. 2B). A few nodule cells of the infection zone were infected by rhizobia. Prominent nuclei and many amyloplasts were also observed in the infection zone. Interzone between infection and nitrogen fixation zones showed transition of nodule cells from uninfected to infected state. Most of the nodule cells in the nitrogen fixation zone contained bacteroids. A large number of bacteroids were arranged around a centrally located large vacuole in each infected nodule cell (Fig. 2C). Lysed nodule cells were seen in the senescence zone.

Loosely packed nodule cells were seen in a section of the nodule induced by the leuC/leuD mutant RS3 (Fig. 2D). A profuse network of infection threads in the intercellular spaces was visible throughout the nodule. Most of the plant cells in the centre of the nodule were flooded with amyloplasts (Fig. 2E). Prominent nuclei were rarely observed in nodule cells. The nodule induced by leuB mutant RH14 showed slightly advanced features over the leuC/leuD mutant induced nodule. Out of five distinct zones of the central tissue of the parental strain induced nodule, meristematic and infection zones were seen (Fig. 2F). Prominent nuclei were visible in many nodule cells in the infection zone (Fig. 2G). The plant cells in the basal half of the nodule contained several amyloplasts (Fig. 2H).

**TEM studies of nodules**—Transmission electron microscopic examination of a section of the nodule induced by the parental strain Rmd201 revealed that the rhizobial cells, which were freshly released from infection thread into plant cells in the infection zone, contained electron dense cytoplasm and poly-β-hydroxy butyrate (PHB) granules (Fig. 3A). Each bacterial cell was enclosed in a peribacteroid membrane (pbm). The bacterial cells in the infection zone were generally spherical. The bacteroids in the nitrogen fixation zone had heterogeneous cytoplasm with electron dense and electron transparent regions (Fig. 3B). These bacteroids were of several shapes but most of these were elongated. The plant cell organelles in the nodule cells of the nitrogen fixation zone took the peripheral position near to the cell wall. Degenerated bacteroids, which had electron transparent cytoplasm and broken pbm, were seen in the senescence zone (Fig. 3C).

There was no release of rhizobial bacteria from infection threads into the cells of the nodule induced by the leuC/leuD mutant (Fig. 3D). Amyloplasts, mitochondria and endoplasmic reticulum profiles were seen in the cells of the nodule. Normal bacterial release from infection threads into the cells of nodule induced by the leuB mutant RH14 was observed (Fig. 3E). The freshly released bacteria were generally spherical. The cytoplasm of these bacteria showed electron dense and electron transparent regions. In
most of the nodule cells the cytoplasm of rhizobial bacteria was almost completely transparent indicating the lysis of these bacteria (Fig. 3F).

Discussion

Six genes, viz., *ilvA*, *ilvB*, *ilvG*, *ilvC*, *ilvD* and *ilvE*, and six genes, viz., *leuA*, *leuC*, *leuD*, *leuB*, *ilvE* and

Fig. 2—Root hair curling and infection thread formation in roots of alfalfa seedlings inoculated with *Sinorhizobium meliloti* Rm201 and its isoleucine + valine auxotrophic mutants. A, Uninoculated. B, C, Rm201 inoculated. D, VK4 inoculated. E, RH1 inoculated. F, RH18 inoculated. Abbreviations: rh-root hair and it-infection thread. Bar, 100 μm (×100).
Fig. 3—Light microscopic studies of longitudinal-semithin sections of nodules induced by *Sinorhizobium meliloti* Rm201 and its leucine auxotrophs. (A) Rm201-induced nodule showing five distinct zones (Mr, If, Iz, Nf & Sc) of the central tissue. Bar, 100μm (X100). (B) Infection zone in Rm201-induced nodule. Bar, 25μm (X400). (C) Nitrogen fixation zone in nodule induced by Rm201. Bar, 25μm (X400). (D) *leuCleuD* mutant RS3-induced nodule showing loosely packed nodule cells. Bar, 100μm (X100). (E) A part of nodule induced by *leuCleuD* mutant RS3 showing a profuse network of infection threads. Bar, 25μm (X400). (F) *leuB* mutant RH14-induced nodule. Bar, 100μm (X100). (G) Infection zone of nodule induced by *leuB* mutant RH14. Bar, 25μm (X400). (H) Nodule cells in the basal half of the nodule induced by *leuB* mutant RH14. Bar, 25μm (X400). Abbreviations: Mr-meristematic zone, If-infection zone, Iz-interzone, Nf-nitrogen fixation zone, Sc-senescence zone, VB-peripheral vascular bundle, it-infection thread, I-infected nodule cell, UI-uninfected nodule cell, B-bacteroid; v-vacuole, Am-amyloplast, n-nucleus & rb-rhizobial bacterium.
Fig. 4—TEM studies of longitudinal-ultramthin sections of nodules induced by *Sinorhizobium meliloti* Rmd201 and its leucine auxotrophs. (A) Freshly released rhizobial cells in the infection region of a nodule induced by Rmd201. Bar, 1 μm (X5, 800). (B) Elongated bacteroids in the nitrogen fixation zone of a nodule induced by Rmd201. Bar, 1 μm (X4, 900). (C) Bacteroids in the senescence zone of a nodule induced by Rmd201. Bar, 1 μm (X6, 800). (D) A part of a cell of a nodule induced by *leuC*/*leuD* mutant RS3. Bar, 1 μm (X5, 300). (E) A part of a cell of a nodule induced by the *leuB* RH14 mutant. Bar, 1 μm (X5, 000) and (F) Cells of a nodule induced by the *leuB* mutant RH14. Bar, 1 μm (X4, 700). Abbreviations: rb-rhizobial bacterium, phb-poly-β-hydroxybutyrate granule, pbm-peribacteroidal membrane, v-vacuole, m-mitochondrion, ER-endoplasmic reticulum, Am-amyloplast, cw-cell wall & B-bacteroid.
tyrB are involved in the biosynthesis of isoleucine and valine, and leucine, respectively, in bacteria. To find out if any one or some of these genes of rhizobia have a direct role in S. meliloti-M. sativa symbiosis, symbiotic characterization of isoleucine and valine, and leucine auxotrophs of S. meliloti Rmd201 strain was done.

All ilvD mutants induced nitrogen fixing nodules on alfalfa plants and nitrogen fixing efficiencies of these mutants were similar to the nitrogen fixing efficiency of the parental strain Rmd201. Aguilar and Grasso9 have also reported normal symbiotic activity of the ilvD mutant of S. meliloti 1021 strain. These results indicate that the expression of ilvD gene does not have a direct role in the symbiosis of S. meliloti with alfalfa plant, and the alfalfa plant is able to provide both isoleucine and valine amino acids to rhizobia during nodule formation and function. All ilvC mutants were Nod and the Nod+ phenotype was not restored by the supplementation of the plant nutrient medium with isoleucine and valine, or α-keto-β-methylvalerate and valine. These results show that the intermediate product(s) α,β-dihydroxy-β-methylvalerate and/or α, β-dihydroxyisovalerate (produced by the ilvC encoded enzyme acetohydroxy acid isomeroreductase) may have a role in nodule formation. Another possibility, according to Aguilar and Grasso9, is that the enzyme acetohydroxy acid isomeroreductase in S. meliloti is able to recognize a substrate (which is not a part of isoleucine and valine pathways) and the product of this substrate is required for nodule formation.

Plant flavonoids induce the expression of rhizobial nod genes and the products of nod genes synthesize a Nod factor which results in the deformation and curling of root hairs, and division of cortical cells in the roots of the legume plant23-25. Aguilar and Grasso9 have reported that in the ilvC mutant isolated by them nodABC genes were not activated by the inducer luteolin and hence Nod factor was not formed. The ilvC mutants isolated during our work appear to synthesize, in response to plant signal, a defective Nod factor since these mutants were found to induce root hair curling. Recently Lópes and coworkers15 have found that ilvC mutants obtained from different S. meliloti wild type strains were able to induce root hair deformation. Variable activation of the common nodulation genes nodABC was observed in these mutants. As no root hair curling was noticed in the single ilvB/ilvG mutant, the expression of ilvB and/or ilvG gene(s) also seems essential for the synthesis of a functional Nod factor.

S. meliloti strain Rmd201 used in this study is a derivative of strain AK631 which in turn is a compact colony mutant of genetically well-characterized strain Rm41. No ilv gene has been located on the chromosomal map of S. meliloti Rm41 strain26, 27. In this work ilvB/ilvG, ilvC and ilvD genes have been found to be linked to ade-15 marker.

All leucine auxotrophs of S. meliloti isolated by earlier workers were Nod+ Fix-11-13. The leucine auxotrophs of S. meliloti isolated during our work were also nod+ fix-. These findings suggest that either the alfalfa host does not provide leucine to rhizobial bacteria during symbiosis or a specific enzyme(s) and/or intermediate(s) of leucine biosynthetic pathway of S. meliloti has (have) a direct role in symbiosis. The supplementation of leucine to the plant nutrient medium did not restore symbiotic effectiveness to the leucine auxotrophs. This result shows that either leucine from the plant nutrient medium is not reaching rhizobia inside the nodules or a specific enzyme(s) and/or intermediate(s) of leucine biosynthetic pathway of S. meliloti is (are) directly involved in symbiosis. The uptake problem for leucine seems unlikely since Truchet et al14 have reported the restoration of nitrogen fixation in the nodules induced by leucine auxotrophs by supplementing leucine in the plant nutrient medium. During this work nitrogen fixation was not restored in the nodules induced by leucine auxotrophs in the α-ketoisocaproate supplemented plant medium indicating that leuCl-leuD and leuB genes may have a direct role in symbiosis.

Histological studies revealed that the nodules induced by the leucine auxotrophs did not develop fully like those induced by the parental strain. The nodules induced by leuB mutants were structurally more advanced than the leuCl-leuD mutant induced nodules. In the former case rhizobial bacteria were released from the infection threads into plant cells whereas in the latter case the rhizobia did not come out of the infection threads. The reason for this result may be that leuC and leuD encoded enzyme isopropylmalate dehydratase converts an unknown substrate into a product which is required for the release of rhizobial bacteria from the infection threads into plant cells. Another reason for the above result may be that the leucine biosynthetic pathway intermediate β-isopropylmalate, which is formed by the action of isopropylmalate dehydratase, has a role in rhizobial release into plant cells. Other workers11, 13 have reported that in the nodules induced by leucine
auxotrophs release of rhizobia from the infection threads did not occur. However the positions of biochemical blocks in these auxotrophs were not known.

Among the plants inoculated with leucine auxotrophs, a few plants had nodules containing prototrophic revertants. The revertant rhizobial cells formed as a result of spontaneous excision of transposon Tn5 obviously had a selective advantage over the auxotrophic cells in nodule formation because the frequencies of nodule occupancy by the revertants were much higher than the frequency of spontaneous excision of Tn5.

One hundred per cent co-transfer of Tn5 and isoleucine-valine, and leucine auxotrophs indicated complete linkage between transposon Tn5 insertion and isoleucine-valine and leucine auxotrophs, respectively. Prototrophic revertants of each auxotroph showed normal symbiotic activity with alfalfa plants. These findings revealed that a single Tn5 insertion in each auxotroph resulted in auxotrophy and symbiotic defect.

The results of this work suggest that ilvB/ilvG, ilvC and one or two leu genes of S. meliloti may have a role in symbiosis. Further work on the symbiotic role of the rhizobial genes of isoleucine, valine and leucine biosynthetic pathways is expected to help in getting better understanding of the complex association between rhizobia and legume plants.

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References


