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Genetic Mapping of Leucine and Isoleucine-Valine Loci in *Rhizobium japonicum*

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Leucine and isoleucine-valine loci have been mapped in *Rhizobium japonicum*. Transformation analysis suggests a common pathway for isoleucine-valine biosynthesis. Three-point reciprocal crosses indicate that all the leucine loci are not genetically linked.

Transformation and transduction have been well established in rhizobia, mainly by using antibiotic resistance markers (1, 3, 5, 9). These studies have not been extended further for the mapping of the *Rhizobium* genome. In our laboratory we have isolated some auxotrophic mutants of *Rhizobium japonicum* and showed a linkage between *argD* and *pyrA* loci by transformation and transductional analysis (2). The present report represents the mapping of some of the leucine and isoleucine-valine loci. The auxotrophic mutants, listed in Table 1, were isolated from *R. japonicum* D-211^S using *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Aldrich Chemicals) as a mutagenic agent. Composition of the media for the growth and development of competence and the deoxyribonucleic acid extraction procedure have been described (2, 6). Five independently isolated leucine auxotrophs were transformed by the deoxyribonucleic acid obtained from the wild-type strain. Reciprocal transformations were also performed between all the possible combinations among *leu* mutants. The results of these crosses (Table 2) clearly suggest that only three of the *leu* mutational sites, i.e., F 81, F 6, and F 99, are linked. F 98 and F 90 did not show any linkage.

Three independent isolates of isoleucine-valine-requiring auxotrophs were transformed by deoxyribonucleic acid from the wild type (Table 3). No *val*$^+$ transformants were obtained when F 40 and F 41 were plated on the minimal medium containing isoleucine. The basis of this inhibitory effect of isoleucine is not known. However, all *ile*$^+$ transformants tested also acquired the *val*$^+$ allele, indicating mutations of

TABLE 1. *R. japonicum strains used for transformation and transduction*[a]

Strain	Genotype
R. japonicum D-211^S	Wild type
F 6	*leu*$^-$
F 81	*leu*$^-$
F 99	*leu*$^-$
F 98	*leu*$^-$
F 90	*leu*$^-$
F 68	*ilv*$^-$
F 40	*ilv*$^-$
F 41	*ilv*$^-$

[a] All auxotrophic mutants were isolated from *R. japonicum* D-211^S by using *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) as a mutagenic agent in the laboratory. An overnight growth culture was centrifuged and suspended in fresh complex medium. After 2 h of incubation on a shaker at 30 C, NTG (100 μg/ml) was added and the culture was incubated further for 45 min. After 45 min, cells were collected by centrifugation, washed thrice with normal saline (0.15 M NaCl), suspended in fresh complex medium, and incubated for 2 h. Subsequently, two NTG treatments (150 and 200 μg/ml, respectively) were given and, after thorough washing (three to four times) with normal saline, cells were suspended in fresh complex broth and incubated overnight on a shaker at 30 C. The mutants were characterized by the replica plating technique.

TABLE 2. *Transformation frequency of leucine auxotrophs*

Recipient strain	Transformation frequency (%)					
	Wild type[a]	F 6[a]	F 81[a]	F 99[a]	F 98[a]	F 90[a]
F 6	2.1×10^{-3}		5.8×10^{-4}	6.4×10^{-4}	2.1×10^{-3}	2.0×10^{-3}
F 81	1.9×10^{-3}	5.7×10^{-4}		7.6×10^{-4}	1.9×10^{-3}	2.1×10^{-3}
F 99	1.8×10^{-3}	6.5×10^{-4}	7.5×10^{-4}		1.7×10^{-3}	2.4×10^{-3}
F 98	1.5×10^{-3}	2.0×10^{-3}	1.8×10^{-3}	1.6×10^{-3}		1.4×10^{-3}
F 90	1.8×10^{-3}	1.9×10^{-3}	2.0×10^{-3}	2.3×10^{-3}	1.8×10^{-3}	

[a] Donor strain.

TABLE 3. *Transformation frequency of isoleucine-valine auxotrophs*

Recipient strain	Selected marker	No. of transformants tested	No. carrying unselected marker
F 68	*ile*$^+$	100	*val*$^+$, 100
	val$^+$	100	*ile*$^+$, 100
F 40	*ile*$^+$	100	*val*$^+$, 100
F 41	*ile*$^+$	100	*val*$^+$, 100

TABLE 4. *Transformation frequency of isoleucine-valine auxotrophs*

Recipient strain	Transformation frequency (%)			
	Wild type[a]	F 68[a]	F 40[a]	F 41[a]
F 68	3.6×10^{-3}		1.6×10^{-3}	1.1×10^{-3}
F 40	4.0×10^{-3}	1.9×10^{-3}		2.0×10^{-3}
F 41	5.0×10^{-3}	1.7×10^{-3}	2.6×10^{-3}	

[a] Donor strain.

the *ilv* type. Reciprocal transformations among isoleucine-valine auxotrophs were performed (Table 4). All three isoleucine-valine auxotrophs showed considerable linkage.

Transformation data clearly suggest that leucine loci are not clustered in one region in *R. japonicum*, unlike *Escherichia coli* and *Salmonella typhimurium*, in which the genes specifying the leucine biosynthesis are clustered in one region and comprise an operon (8, 7). The studies on isoleucine-valine auxotrophs indicate that there might be a common pathway for isoleucine-valine biosynthesis in *R. japonicum* similar to the ones found in *E. coli* and *S. typhimurium*.

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