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Carbon Utilization and Regulation of Nitrogen Fixation Genes in *Rhizobium meliloti*

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A *dctB* mutant (CM12) and a *dctBD* mutant (CM20) of *Rhizobium meliloti* displaying Fix$^-$ and Fix$^+$ phenotypes, respectively, were used to investigate the expression of nitrogen fixation genes during symbiosis. The levels of expression of the *nifHDK* promoter (P1), the *fixABCX* promoter (P2), and the *nifA* promoter (P*nifA*) were monitored during symbiosis and under free-living microaerobic conditions. The levels of expression in nodules formed by *R. meliloti* CM12 were less than 10% of the levels found in nodules formed by wild-type *R. meliloti* (strain CM2). Levels in nodules formed by CM20 were similar to those in wild-type cells. Under free-living microaerobic conditions, the level of expression of nitrogen fixation gene fusions in the wild-type *R. meliloti* CM2 varied strongly on different carbon sources used in the growth media. The highest levels of expression of P*nifA* and P1 were detected on dicarboxylic acids and on mannitol; the lowest were observed on glutamate. Glucose supported intermediate levels of expression. When mannitol was exploited as an alternative carbon source to support growth of the *dct* mutants, P*nifA* was found to be expressed at wild-type levels in both CM12 and CM20. In CM20, expression of P*nifA* resulted in wild-type levels of P1 expression. In CM12, however, this was not the case. The possibility of a regulatory link between the expression of *R. meliloti nifHDK* genes and the activity of the regulatory *dct* genes is discussed.

In the *Rhizobium*-legume symbiosis, the energy needed for nitrogen fixation is ultimately derived from the photosynthate supplied by the plant to the bacteroid. Evidence indicates that photosynthate availability may be a limiting factor for the nitrogen-fixing capacity of the bacteroid (Hardy and Havelka 1975; Ryle *et al.* 1979). It is now generally accepted that the energy for nitrogen fixation is derived principally from C_4-dicarboxylic acids (dCA: succinate, malate, and fumarate). Mutants of *R. meliloti* (Bolton *et al.* 1986; Engelke *et al.* 1987; Watson *et al.* 1988), *R. leguminosarum* (Arwas *et al.* 1985; Finan *et al.* 1983; Glenn and Brewin 1981), and *R. trifolii* (Ronson *et al.* 1981) that are unable to transport dCA, but capable of utilizing other carbon sources, generally form ineffective nodules on their respective host plants. Also, dCA are rapidly taken up by isolated bacteroids (Reibach and Streeter 1984) and support high rates of respiration and nitrogenase activity (Bergersen and Turner 1967; Miller *et al.* 1988).

Work on the regulation of the dCA transport (Dct) system suggests that succinate, malate, and fumarate are transported actively via a common transport system in both cultured cells and bacteroids of *Bradyrhizobium japonicum* (Humbeck and Werner 1987; McAllister and Lepo 1983; San Francisco and Jacobson 1985), *R. meliloti* (Engelke *et al.* 1987), and *R. leguminosarum* (Finan *et al.* 1981; Glenn *et al.* 1980). In *R. leguminosarum* a cluster of three genes has been identified that is specifically required for Dct in the free-living bacterium (Ronson and Astwood 1985; Ronson *et al.* 1984; Ronson *et al.* 1987a). The structural gene (*dctA*) is required for transport in both symbiotic and free-living *R. leguminosarum* (Ronson *et al.* 1987a). *dctB* and *dctD* encode a two-component regulatory system that is required for the expression of *dctA* in the free-living bacterium in response to the presence of dCA.

In the proposed model, DctB is the sensor located in the cell membrane. In response to the presence of dCA in the environment, DctB modulates the intracellular mediator (DctD), which then will turn on *dctA*. The product of the general nitrogen regulatory gene (*ntrA*) is also required for expression of *dctA* (Ronson *et al.* 1987b). It appears that the *dctA* gene is regulated differentially in the free-living bacterium and during symbiosis since some strains that are mutated in the regulatory *dct* genes form nodules that fix nitrogen at rates similar to the wild type (Ronson *et al.* 1987a; Ronson 1988; Wang *et al.* 1989; Watson *et al.* 1988). These Fix$^+$ *dct* mutant strains apparently do transport dCA during symbiosis since *dctA*::*lacZY* fusions are expressed to wild-type levels in the nodule (Ronson 1988; Wang *et al.* 1989). The Fix$^-$ phenotype of most *dct* mutants is generally attributed to the lack of an adequate energy supply in the bacteroid to support nitrogenase activity. However, investigations have not previously focused on whether *nif* genes are expressed in bacteroids whose main energy supply has been disrupted.

In our laboratory, we are investigating the role of dCA in the *Medicago sativa* L.-*R. meliloti* symbiosis, and here we report on the expression of nitrogen fixation genes in relation to the presence or absence of an active Dct system during symbiosis.

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MATERIALS AND METHODS

Bacterial strains. The bacterial strains and plasmids used in this study are listed in Table 1.

Aerobic growth. Complex media were Luria-Bertani (Miller 1972) for *Escherichia coli* and MSY (O'Gara and Shanmugam 1976) for *R. meliloti*. Minimal salts medium for *R. meliloti* was described by O'Gara and Shanmugam (1976).

Microaerobic growth. Cultures were grown microaerobically using a modification of the nitrogenase induction conditions routinely used for *B. japonicum* cultures (Birkenhead *et al.* 1988). Aerobically grown cells were washed in carbon- and nitrogen-free minimal salts and used to inoculate (1%) the minimal salts medium (2 ml in 71-ml serum bottles) containing 0.4% (w/v) as the carbon source and 0.1% (w/v) glutamate as the nitrogen source. Initially the gas phase in the assay flasks was 100% argon.

The cultures were incubated at 30° C and the shaker speed was approximately 160 rpm (orbital shaker, LM Engineering Co., Bucks, England). After 24 hr of incubation, at 24-hr intervals, oxygen was added to the gas phase with a Hamilton syringe in sufficient amounts to achieve the desired pO_2. In some experiments, the carbon source and the concentration of oxygen were varied. Such changes are noted in the text. Growth was followed by measuring the optical density at 600 nm. β-Galactosidase (βGal) assays were performed at 24-hr intervals over a 5-day period, as described by Miller (1972).

The expression of *nif* fusions in *R. meliloti* is very much dependent on the oxygen tension in the culture medium (Ditta *et al.* 1987; O'Gara *et al.* 1988). Therefore, the optimum concentration for maximum expression of the P1 and P*nifA* fusions was determined by measuring βGal activity in CM2(pMB210) and CM2(pCHK57) during growth in 0.4% malate as the carbon source and 0.1% glutamate as the nitrogen source over a range of oxygen concentrations between 0.76 and 760 mm Hg. Maximum levels of P1 expression were detected with an oxygen concentration of 0.76 mm Hg during mid-logarithmic growth. A similar concentration of oxygen resulted in maximum expression of P*nifA* in CM2(pCHK57), but this occurred before cells entered the logarithmic growth phase. This concentration of oxygen was used in subsequent experiments unless otherwise indicated.

Plant growth. Alfalfa seeds (*M. sativa* cv. Du-Puits) were surface-sterilized and germinated as previously described (Wang *et al.* 1989). Two-day-old seedlings were transferred to large test tubes containing carbon- and nitrogen-free rooting solution (Wang *et al.* 1989) and inoculated with the appropriate *R. meliloti* strain. Plants were grown in a greenhouse with tungsten lighting for 16 hr per day (10,000 lx; minimum temperature 14° C and maximum temperature 22° C). To determine βGal activity from nodules, the nodules from 10–15 plants were transferred to a test tube containing 1.0 ml of cold 250 mM mannitol, 50 mM tris(hydroxymethyl)aminomethane (Tris; pH 7.8). The nodules were crushed with a glass rod, and the plant debris was allowed to settle for 15 min on ice before removing bacteroid aliquots for immediate assay. A 0.5-ml aliquot of the bacteroid suspension was added to 0.5 ml of Z buffer (Miller 1972), and βGal activity was measured as described by Miller (1972).

Since βGal expression was plasmid-derived, the stability of the plasmids in nodules was determined. Nodules were surface-sterilized for 30 s in methanol and 2 min in 20% (v/v) H_2O_2, washed three times in sterile distilled water, and crushed in Ringer's solution. Bacteria isolated from the nodules were screened for tetracycline resistance that was encoded by the vector plasmid. The percentage of tetracycline-resistant cells per nodule ranged from 51 to 79%, and averaged 62%.

Sample preparation for microscopy. Whole nodules were photographed using a Wild M8 zoom stereomicroscope.

Nodules were fixed under reduced pressure for 3 hr in 4% (w/v) glutaraldehyde in 200 mM sodium cacodylate (pH 7.2), washed in buffer for 3 hr, and postfixed in 1% (w/v) OsO_4 in the same buffer under reduced pressure. Samples were dehydrated in acetone, infiltrated for 48 hr, and embedded in Epon 812 resin. Semithin (1 μm) and ultrathin (600 Å) sections were cut on the LKB Ultramicrotome III using glass knives, stained with uranyl acetate and lead citrate (Reynolds 1963), and examined in the JEM P200EX electron microscope. The semithin sections were examined at an accelerating voltage of 120 kV and were used for lower magnification survey photographs. The ultrathin sections were examined at 80 kV and were mainly used for higher magnification photographs.

Plasmid transfer. The plasmids listed in Table 1 were transferred into *R. meliloti* strains from *E. coli* by conjugation. The frequency of tetracycline resistance transfer was between 5.2×10^{-5} and 9.5×10^{-5}. All matings were triparental and pRK2013 was used as the mobilizing plasmid. Matings were conducted on MSY medium at 30° C

Table 1. Bacterial strains and plasmids used in this study

Designation	Relevant characteristics[a]	Source or reference
Rhizobium meliloti		
CM2	Wild type, Rif^r	Manian and O'Gara 1982
CM12	*dctB* Rif^r, NTG derivative of CM2	Bolton *et al.* 1986
CM20	*dctBD* Rif^r, Tn*5* derivative of CM2	Bolton *et al.* 1986
Escherichia coli		
HB101	*recA, hsdR, hsdM, strA, pro, leu, thi*	Boyer and Roulland-Dussoix 1969
MC1061	*ara, leu, lac*ΔX74, *galU, galK, hsdR, hsdM, strA*	Casadaban and Cohen 1980
Plasmids		
pRK2013	ColE1, Mob$^+$, Tra$^+$ (RK2), Km^r	Figurski and Helinski 1979
pGD926	pRK290::*lacZY*, Tc^r	Ditta *et al.* 1985
pMB210	pGD926::P1, Tc^r	Better *et al.* 1985
pMB211	pGD926::P2, Tc^r	Better *et al.* 1985
pCHK57	pGD926::P*nifA*, Tc^r	Ditta *et al.* 1987
pCUH3	pSUP102::*dctABD*, Cm^r	Wang *et al.* 1989
pCU24	pCUP1::*Rm dctA*::*lacZY*, Gm^r	Wang *et al.* 1989

[a] Rif, rifampicin; Km, kanamycin; Tc, tetracycline; Cm, chloramphenicol; Gm, gentamycin; ^{r}, resistant; and NTG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

for 48 hr. Exconjugants were selected on MSY medium containing tetracycline (10 μg/ml) and rifampicin (100 μg/ml).

Protein assay. Total protein was determined by the method described by Lowry *et al.* (1951), and bovine serum albumin was used as a standard.

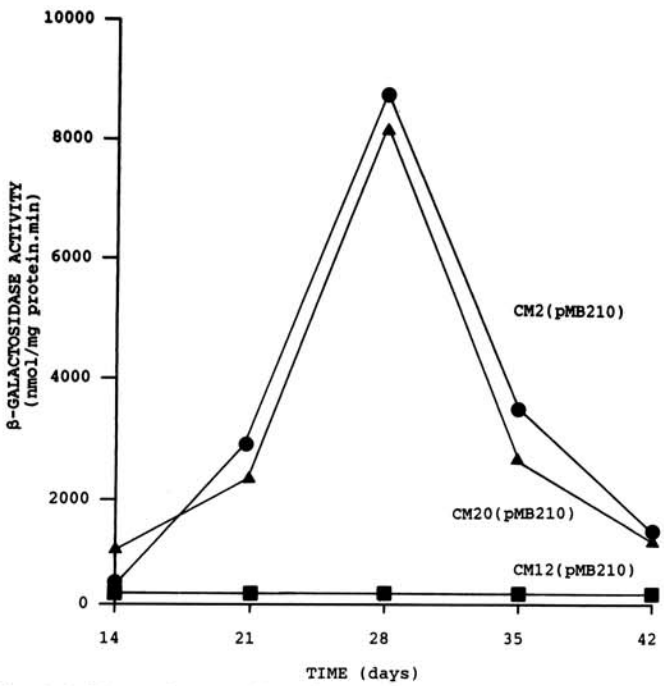

Fig. 1. β-Galactosidase activity of pMB210 (P1) containing bacteroids isolated from CM2, CM12, and CM20 nodules over a 5-wk period. The nodules from 5–10 plants were transferred to a test tube containing 1.0 ml of 250 mM mannitol, 50 mM Tris-HCl, pH 7.8. The nodules were crushed, and the plant debris was allowed to settle before removing bacteroid aliquots for immediate assay. β-Galactosidase assays were performed as described by Miller (1972). Each point is the average of at least three independent measurements.

RESULTS AND DISCUSSION

Regulation of *R. meliloti* symbiotic promoters in *dct* mutant strains. To investigate the effect of a defective Dct system on the expression of *nif* genes during symbiosis, the expression of several symbiotic promoters in nodules formed by regulatory *dct* mutants was studied. We selected an *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-induced *dctB* mutant of *R. meliloti* CM2 (strain CM12) that showed no detectable nitrogenase activity during symbiosis (Fix^-) and a Tn*5*-induced *dctBD* mutant (strain CM20) that has a Fix^+ phenotype (Wang *et al.* 1989). The plasmid pMB210, which contained a translational fusion of the *nifH* gene to *lacZY* (in pGD926), was introduced into the wild-type strain CM2 and the *dct* mutant strains CM12 and CM20. βGal activity of bacteroids containing pMB210 was determined following isolation from alfalfa nodules between 2 and 7 wk after inoculation (Fig. 1). Nodules formed by CM2 showed high levels of expression of the *nifHDK* promoter (P1) from week three to week seven, with maximum levels of expression being observed in 4-wk-old nodules. Bacteroids from nodules induced by CM20 showed levels of P1 expression similar to those in wild-type strain CM2. However, there was no significant level of P1 expression detected in CM12-induced bacteroids (less than 2% of wild-type levels from week three onwards).

CM2- and CM20-induced nodules were large, pink, and relatively few in number. In contrast, nodules formed by CM12 were typical of a Fix^- phenotype, that is small, white nodules that were three to five times as numerous (Fig. 2). Ultrastructural studies of 3-wk-old nodules showed that CM12-induced nodules did contain bacteroids. Nodule development and bacteroid differentiation appeared similar to that in the wild-type CM2 nodules (Fig. 3). Therefore, it appears unlikely that the lack of P1 expression in bacteroids isolated from CM12 nodules was caused by early senescence of the bacteroids at the time of assay. Neither

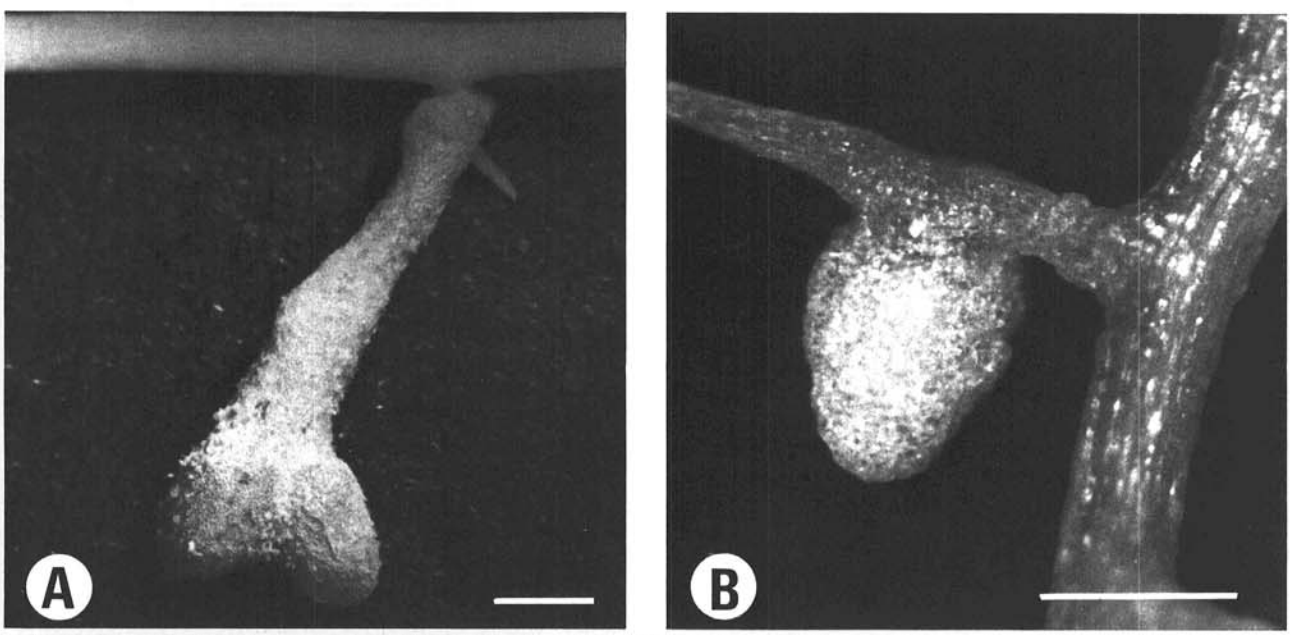

Fig. 2. Light micrograph of 42-day-old nodules induced by *Rhizobium meliloti* wild-type strain CM2 (**A**) and *dct* mutant CM12 (**B**). Bars = 1 mm.

can it be explained through plasmid loss, since the average number of tetracycline-resistant cells recovered from 6-wk-old CM12-induced nodules was similar to the plasmid retention frequency for CM2 nodules.

Based on the failure to detect significant expression of P1 in CM12, it was decided to investigate the expression of other symbiotic promoters. It was of particular interest to see whether the promoter of the *fixABCX* genes (P2)

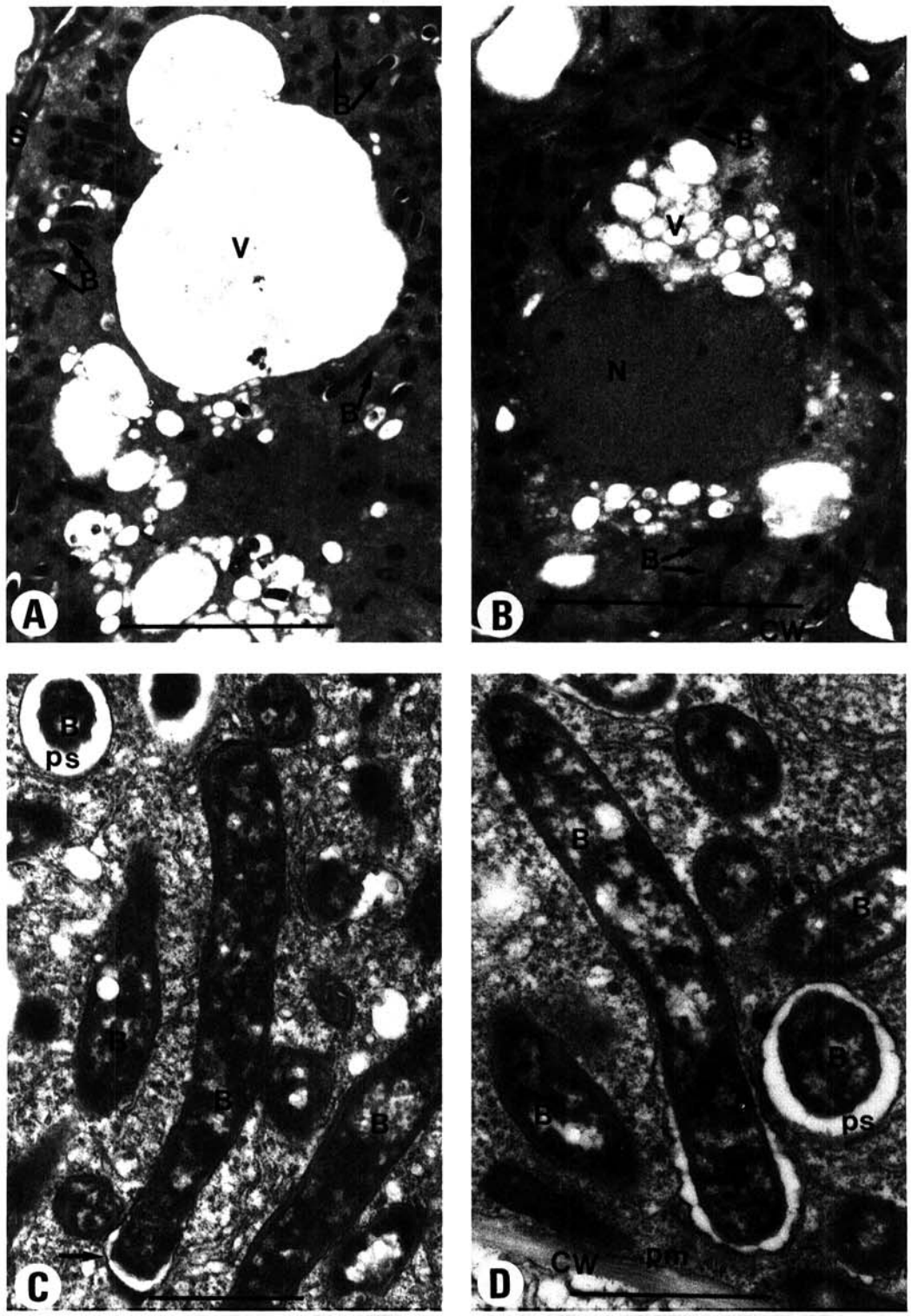

Fig. 3. **A** and **B**, Electron micrographs of a 1-μm section through a 20-day-old wild-type (CM2) induced nodule (**A**) and a *dctB* mutant (CM12) induced nodule (**B**). The micrographs show a significant portion of individual host cells containing bacteroids (B), nucleus (N), starch grains (S), and vacuoles (V). The cell wall (CW) between the host cells is clearly seen. Bars = 10 μm. **C** and **D**, Electron micrographs of ultrathin sections showing a detailed view of CM2 (**C**) and CM12 (**D**) bacteroids. Bacteroids are surrounded by a host-derived peribacteroid membrane (arrow). The peribacteroid space (ps) is clearly visible in some cases. The host cell plasma membrane (pm) and cell wall (CW) can be seen. Bars = 1 μm.

and the promoter of the regulatory *nifA* gene (P*nifA*), whose gene product is known to be required for transcriptional activation of both P1 and P2 (Szeto *et al.* 1984), would also be affected in a similar manner. The patterns of expression of P2 (pMB211) and P*nifA* (pCHK57) in CM2, CM12, and CM20 bacteroids were similar to those obtained with P1. The levels of the βGal activity detected for these fusions in 4-wk-old alfalfa nodules are presented in Table 2. Bacteroids formed by CM20 always showed promoter activity similar to that in the wild type. In contrast, bacteroids from nodules formed by CM12 showed less than 10% of the βGal levels of P2 and P*nifA* observed in the wild-type strain, CM2. Complementation of CM12 with the *dct* operon restored the expression of all the *nif* and *fix* gene fusions (Table 2).

Expression of *dctA* in *dct* mutant strains. The expression of the *dctA* promoter was also monitored in nodules during symbiosis. The *dctA* promoter was expressed in nodules formed by CM20 to about 60% of the wild-type level, even though the regulatory *dct* genes were mutated (Table 2). It has been suggested that an alternative symbiotic activator is responsible for activating the *dctA* promoter during symbiosis (Ronson *et al.* 1987a). This activation of the *dctA* promoter during symbiosis apparently does not function in CM12 since the expression of the *dctA* fusion is severely impaired. CM12 is a *dctB* mutant and the *dctD* gene product (DctD) is presumably present, but cannot be modulated by DctB. DctD, therefore, remains in an inactive form. This inactive DctD may bind to the *dctA* promoter and prevent induction by the alternative symbiotic activator (Ronson *et al.* 1987a). In CM20, the *dctA* promoter is expressed in the nodule since the polar effect of the Tn*5* mutation in *dctB* blocks the production of the DctD as well as DctB (Wang *et al.* 1989).

dCA are considered to be the major carbon source supplied by the plant to the bacteroid. Since the *dctA* promoter is expressed in CM20 during symbiosis, but not in CM12, the lack of expression of the symbiotic promoters in CM12 could be attributed solely to a limited energy supply. However, two observations indicate that this is not the only possible explanation. First, nodule development appears to be normal, and this would indicate that alternative carbon sources must be available to the bacteroid to sustain a certain level of gene expression. Second, a number of constitutive nonsymbiotic promoters, such as an *E. coli* drug-resistance promoter (pGD139, this laboratory) and a *Rhizobium* haem biosynthetic promoter (pXLGD4, Leong *et al.* 1985), were found to be expressed in CM12-induced nodules to about 60% of the wild-type levels (data not shown). This is in contrast with the almost complete suppression of the symbiotic promoters in the CM12-induced nodules.

Regulation of *R. meliloti* symbiotic promoters by the available carbon source. To further investigate the argument of a limited carbon supply that affects *nif* gene expression, it was decided to exploit the asymbiotic assay system (Ditta *et al.* 1987; O'Gara *et al.* 1988) to study *nif* gene expression. This allows the expression of symbiotic promoters to be monitored in *R. meliloti* under free-living conditions. Unlike the symbiotic system where the bacteroid depends on the carbon provided by the plant host, it is possible to vary the carbon source in the asymbiotic assay system. Therefore, the activity of the symbiotic promoters can be monitored in *dct* mutant strains growing on carbon sources other than dCA. To determine a suitable alternative to dCA, *nif* gene expression in the wild-type strain CM2 was monitored on various carbon sources.

The growth of CM2(pCHK57) and CM2(pMB210) and the level of P*nifA* and P1 expression were monitored on minimal media containing a variety of different carbon sources (0.4% [w/v]). Glutamate (0.1% [w/v]) was used as the nitrogen source, and the oxygen tension was 0.76 mm Hg. No significant differences were evident in the specific growth rates of the strains on the various carbon sources used (mannitol, malate, glucose, and glutamate; Table 3). This indicates that all these carbon sources are equally effective in sustaining the growth of CM2. However, the level of expression of the symbiotic promoters varied significantly on the various carbon sources. High levels of P*nifA* and P1 activity were obtained on either 0.4% malate or 0.4% mannitol (Table 3). These levels were comparable to those obtained in the bacteroids (Table 2). Glucose supported intermediate levels of P*nifA* and P1 activity. When the wild-type strain CM2 was grown on 0.5% glutamate as the sole carbon and nitrogen source, low levels of βGal were detected for P*nifA* and P1 (less than 5% of the levels detected on malate or mannitol for P1). Varying the concentration of oxygen between 0.076 and 2 mm Hg did not significantly improve the low levels of expression of P*nifA* and P1 on glutamate. These results show that the expression of P*nifA*, and consequently P1, is influenced by the nature of the carbon source available. It is not clear how this effect is mediated, and it will be interesting to determine if *fixLJ* genes are involved. The

Table 2. Expression of symbiotic and nonsymbiotic *lacZY* fusions in 28-day-old nodules formed by *Rhizobium meliloti* strains

Plasmid	Promoter	β-Galactosidase activity (nmol/min/mg of protein) of[a] CM2 (wild-type)	CM12 (*dctB* mutant)	CM20 (*dctBD* mutant)	CM12pCUH3	Activity of CM12 (As a % of wild-type value)	Activity of CM20 (As a % of wild-type value)
pMB210	P1(*nifHDK*)	8,693 ± 712	121 ± 26	8,432 ± 418	8,312 ± 690	1	96
pMB211	P2(*fixABCX*)	7,752 ± 546	136 ± 32	7,519 ± 346	7,816 ± 493	2	97
pCHK57	P(*nifA*)	3,417 ± 217	102 ± 16	2,733 ± 411	3,253 ± 304	2	80
pCU24	P(*dctA*)	293 ± 32	27 ± 8	195 ± 47	324 ± 41	9	66
pGD926	None	21 ± 3	17 ± 5	20 ± 1	25 ± 9		

[a] β-Galactosidase activity was measured in bacteroids isolated from 28-day-old alfalfa nodules as described in the legend of Figure 1. Each value is the mean of at least five replicates, and the standard error is given.

fixLJ genes are considered to respond to the oxygen tension and under low oxygen tension positively induce P*nifA* expression (David *et al.* 1988).

The mutation of the *dctB* gene in CM12 prevents expression of the *dctA* gene during symbiosis and thereby the uptake of dCA. The bacteroid, therefore, has to rely on alternative carbon sources, and as shown above, not every source is equally effective in sustaining the expression of P*nifA*. (For instance, if glutamate was the only available carbon source in CM12-induced nodules, we would expect P*nifA* to be severely repressed [Table 3].) This could explain the low levels of P*nifA* expression in CM12-induced nodules (Table 2). A low level of NifA would in turn result in a low level of P1 and P2 expression (Szeto *et al.* 1984).

A regulatory link between the *dct* operon and the *nifHDK* promoter. Since similar levels of symbiotic promoter activity were detected in CM2 during growth on either malate or mannitol, it was decided to monitor P*nifA*, P1, and P2 expression in the *dct* mutant strains during microaerobic growth on mannitol as an alternative carbon source to dCA. This resulted in the *nifA* promoter being expressed at high levels in both *dct* mutants (Table 4). In CM20, expression of NifA led to induction of P1 and P2. In CM12, however, this was not the case since wild-type levels of P2 were detected, but P1 was not induced (Table 4). This block on the *nifHDK* expression in CM12 was alleviated by complementation with the cloned *dct* operon on pCUH3 (Table 4). Complementation of CM12 with pCUH3 also resulted in restoration of nitrogen fixation during symbiosis (data not shown). These findings indicate that the mutation in the *dctB* gene of CM12 has an effect on the expression of the *nifHDK* genes under microaerobic conditions. This effect was not exerted through NifA, since the levels of P*nifA* expression were as high as in the wild type, but appeared to act directly on P1. P2 was normal too, proving that NifA was functional.

A possible explanation for this block in P1 may be that an inactive DctD interferes with the expression of this promoter, analogous to the way it is proposed to interfere with the expression of *dctA* during symbiosis. We are currently investigating the possibility of DctD acting this way on the *nifHDK* promoter. Also, the block on the *nifHDK* promoter in *Rhizobium* strains in which the *dctD* is turned into a repressor, for example CM12, *R. meliloti* 5421 (Yarosh *et al.* 1989), and *R. leguminosarum* 659 (Ronson *et al.* 1987a), appears to be peculiar to these *dct* mutant strains. It is not simply a result of DctD not being activated in these mutant strains since in CM2, in the absence of dCA, the intermediate expression of NifA is followed by corresponding levels of NifH expression (during microaerobic growth on mannitol and glucose, Table 3). Under these conditions, the block on the P1 clearly does not exist.

Table 3. Expression of *nif*::*lacZY* fusions in *Rhizobium meliloti* cultures under free-living microaerobic conditions[a]

	Specific growth rate (h^{-1}) of	β-Galactosidase activity (nmol/min/mg of protein) of	
Carbon source	**CM2**	**CM2(pMB210)**	**CM2(pCHK57)**
Malate	0.062 ± 0.003	8,269 ± 997	1,753 ± 434
Mannitol	0.061 ± 0.004	8,173 ± 842	2,007 ± 197
Glucose	0.050 ± 0.004	3,968 ± 653	1,089 ± 49
Glutamate (0.5%)	0.054 ± 0.005	418 ± 126	393 ± 43

[a]The cultures were grown under microaerobic conditions with various carbon sources (0.4% [w/v]), glutamate (0.1% [w/v]) as the nitrogen source, and an oxygen concentration of 0.76 mm Hg (0.1% [v/v]). β-Galactosidase assays were performed at 24-hr intervals over a 5-day period. The maximum levels of β-galactosidase detected for each fusion are shown in the table. Each value is the mean of at least four replicates, and the standard error is given.

Table 4. β-Galactosidase activity from *nif*::*lacZY* fusions in *dct* mutants of *Rhizobium meliloti* under free-living microaerobic conditions[a]

	Plasmid		
***R. meliloti* strain**	**pMB210(P1)**	**pMB211(P2)**	**pCHK57(P*nifA*)**
CM20	7,132 ± 366	3,041 ± 319	2,106 ± 54
CM12	161 ± 52	3,712 ± 146	2,081 ± 165
CM12(pCUH3)	5,857 ± 304	2,945 ± 213	1,035 ± 29

[a]The cultures were grown under microaerobic conditions with mannitol (0.4% [w/v]) as a carbon source, glutamate (0.1% [w/v]) as the nitrogen source, and an oxygen concentration of 0.76 mm Hg (0.1% [v/v]). β-Galactosidase activity was determined as described in the footnote of Table 3. Each value is the mean of at least four replicates, and the standard error is given.

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