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Expression of nodC Gene in *Rhizobium meliloti* Strains of Different Symbiotic Efficiency

Wyrażenie genu nodC w szczepach *Rhizobium meliloti*
o różnej efektywności symbiozy

Bacteria of genus *Rhizobium* invade specific host plants and stimulate the development of root nodules that are centers of nitrogen fixation. In *Rhizobium meliloti* genes required, for nodulation (nod) and nitrogen fixation (nif) are located on the one of two megaplasmids (1, 4, 9). Among nod genes only nodD gene is expressed constitutively. In the presence of a specific plant molecule the nodD gene product is the transcriptional activator of the other genes involved in the genetic control of nodulation (5, 13). In many *R. meliloti* strains a repressor of nod genes transcription was also detected. It was observed that in repressor-containing strains the nodD gene expressed weakly (6).

Other smaller plasmids in *Rhizobium meliloti* strains may encode functions affecting symbiosis (11). The successive nodulation depends on numerous internal and external factors. The level of nod genes induction also can be influenced by various conditions. The ability to the effective and early nodulation is a very important characteristic of rhizobial strains. They are believed to support the growth of plants infected and increase the chance of nodulation by the particular strain in environmental conditions.

In this paper the differences in the regulation of nod genes expression between two strains of *R. meliloti* are reported.

MATERIALS AND METHODS

Bacterial strains and plasmid are listed in Table 1.

Bacterial matings. The fusion nodC-lacZ was transferred into *R. meliloti* strains in triparental matings. *Escherichia coli* strains harboring pRmM57 and helper plasmid pRK2013 were cultivated in LB medium (7) supplemented with tetracycline (10 μg ml^{-1}) or kanamycine (20 μg ml^{-1}), respectively. *R. meliloti* strains grew in 79CA medium (14). Overnight cultures were centrifuged, washed and concentrated tenfold. 50 μl of donor strains and 100 μl of the recipient *R. meliloti* strain were mixed on 79CA agar slant and incubated in 26°C overnight. Growth of bacteria was next washed with saline and sprayed on 79CA agar plates with tetracycline (10 μg ml^{-1}) and chloramphenicol (15 μg ml^{-1}). *R. meliloti* Bp and Bp17 were naturally resistant to chloramphenicol.

Table 1. List of strains and plasmid used

Strain or plasmid	Characteristic	Reference
Rhizobium meliloti Bp	Nod^+Fix^+	
Bp17	Nod^+Fix^+ the derivative of Bp strain of enhanced symbiotic efficiency	Głowacka (3)
pRmM57	nodC-lacZ fusion in RmSL26 (20kb *R. meliloti* nod gene segment in pLAFR1)	Mulligan, Long (8)
pRK2013	Km^r ColE1 replicon + Tra RK2	Figurski, Helinski (2)

Expression of nodC-lacZ. It was tested on media supplemented with a substrate of β-galactosidase, Xgal (10). Colonies with β-galactosidase activity were blue while others remained white. The level of β-galactosidase activity was assayed using ONPG as a substrate (10). For the induction of nod genes expression the exudate of alfalfa seedlings was used. Surface sterilized alfalfa seeds were germinated and next incubated for 3 days in sterile water at room temperature. Then the exudate was collected and suspended in ethanol.

Plant test. Seeds of alfalfa (*Medicago sativa*) were surface sterilized (3), germinated and placed on nitrogen free agar slants (14). After four days the seedlings were inoculated with 0.2 ml of *Rhizobium* cells suspension in water (10^8 cells ml^{-1}). Plants were cultivated under 14/10 light/dark cycle for 6 weeks.

RESULTS AND DISCUSSION

Rhizobium meliloti Bp17 is a derivative of *R. meliloti* Bp showing enhanced efficiency of symbiotic nitrogen fixation. The green mass of alfalfa plants infected with Bp17 strain was higher than the yield of the green mass of plants inoculated with the parental strain Bp in the laboratory and fields conditions (3). In the repeated plant tests *R. meliloti* Bp17 induced

nodules earlier in comparison with the parental strain. It was suspected that this ability may be a result of changes in regulation of expression of nodulation genes. The level of nodulation rate may be influenced by various factors. Similar effect of increased nodulation ability was observed in four *R. meliloti* strains after introducing of multicopy plasmid carrying *Klebsiella pneumoniae* nifA gene. Probably some other genes had been expressed and increased the nodulation in this case (12).

Table 2. Expression of nodC-lacZ fusion in *Rhizobium meliloti* Bp and Bp17 strains *

Strain	Fusion	Colony number	β-galactosidase activity **	
			−I ***	+I
Bp	nodC-lacZ	1	7.2	15.7
		2	13.3	27.3
Bp17	nodC-lacZ	1	367.5	328.8
		2	9.3	25.0

* Average of four repeats. ** β-galactosidase activity in Miller units (Sambrook et al., 10). *** −I, +I without or with inducer (plant exudate).

To test the possibility of different expression of nod genes in Bp and Bp17 strains the plasmid carrying nodC-lacZ fusion was introduced into both strains. Because nodC is an inducible gene, the level of β-galactosidase activity could point the rate of induction of nod genes. Transconjugants Tc^r of *R. meliloti* Bp and Bp17 were obtained with the frequency 10^{-4} and tested on the medium containing Xgal. Surprisingly, the part of Bp17 Tc^r transconjugants showed blue colour without induction with plant exudate. It suggested that in some cells of Bp17 strain nod genes can express constitutively. Single colonies of Bp: nodC-lacZ (white) as well as Bp17: nodC-lacZ (1 — blue, 2 — white) were quantitatively assayed for β-galactosidase activity (Table 2). In both single colonies of Bp strain and number 2 of Bp17 the inducible expression of nodC-lacZ took place. But in the clone Bp17 number 1 the expression of nodC-lacZ occurred without induction that indicated the changes in regulation of nod genes expression.

It was interesting to know if these changes of regulation influenced the symbiotic efficiency of particular rhizobial clones. For this purpose several single colonies of Bp: nodC-lacZ and both kinds of Bp17: nodC-lacZ were tested in plant test (Table 3). Average weights of plants was highest in the case of plants infected with Bp17: nodC-lacZ number 1, showing an elevated level of nod genes expression without induction. It strongly suggests that regulatory changes of nod genes expression can be the reason of enhanced symbiotic efficiency in *Rhizobium meliloti* Bp17.

Table 3. The yield of plants infected with single colonies of *Rhizobium meliloti* Bp and Bp17

Strain	Number of single colonies tested*	Average weight of plant			
		Total		Green part	
		mg	%	mg	%
Bp: nodC-lacZ	2	129	100	92	100
Bp17: nodC-lacZ					
number 1	3	138	107	101	110
number 2	3	131	102	95	103

* 10 plants were inoculated with each single colony.

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STRESZCZENIE

Szczepy *Rhizobium meliloti* Bp i Bp17 różnią się tempem tworzenia brodawek na korzeniach lucerny oraz wydajnością symbiotycznego wiązania azotu mierzoną przyrostem zielonej masy roślin. Konstytutywne wyrażenie genu nodC w szczepie Bp17 po przekazaniu fuzji nodC-lacZ wskazuje na zmiany regulacji genów kontrolujących brodawkowanie (nod). Klony wykazujące ekspresję nodC bez indukcji wykazywały równocześnie lepszy efekt na wzrost roślin w porównaniu ze szczepem Bp.